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(71) Applicant *(for all designated States except US)*: **DIGITAL GENE TECHNOLOGIES, INC.** [US/US]; Suite 110, 11149 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LEONARDI, Amedi** [IT/IT]; Via Poliziano, 16, I-20154 Milano (IT). **SARTANI, Abraham** [IT/IT]; V. Le Dei Platani, 20/17, I-20020 Arese Milano (IT). **GLASS, James, R.** [US/US]; 1467 Kings Cross Drive, Cardiff-by-the-Sea, CA 92007 (US). **HASEL, Karl, W.** [CH/US]; 247 South Nardo Avenue, Solano Beach, CA 92075 (US).

(74) Agents: **BERLINER, Brian, M.** et al.; O'Melveny & Myers, 400 South Hope Street, Los Angeles, CA 90071-2899 (US).

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(54) Title: MODULATION OF GENE EXPRESSION DURING INTIMAL HYPERPLASIA OF THE CAROTID ARTERY

(57) Abstract: Polynucleotides, polypeptides, kits and methods are provided related to regulated genes characteristic of atherosclerosis.

MODULATION OF GENE EXPRESSION DURING INTIMAL HYPERPLASIA OF THE CAROTID ARTERY

RELATED APPLICATION DATA

The present application claims priority to U.S. Provisional Patent Application No. 60/252,216, filed November 21, 2000, which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Atherosclerosis is a common form of cardiovascular disease and accounts for one-third of all deaths in Western societies. The prevalence of atherosclerosis has led to concentrated efforts to understand the underlying basis of the pathology and to develop effective therapeutics for prevention and treatment. The progression of atherosclerosis in humans often occurs over decades, and can be viewed as a state of chronic inflammation resulting from a cascade of interactions between oxidized or modified lipoproteins, monocyte-derived macrophages, T cells, and the cellular components comprising the arterial wall. This inflammatory process results in the appearance of atherosclerotic lesions, or plaques, that cause thickening of the arteries, loss of vascular elasticity, and thrombus formation. The physiological consequences of these long-term changes manifest themselves in acute myocardial infarction and stroke (Ross, *N Engl J Med*, 1999; Navab et al, *Arterioscler Thromb Vasc Biol,* 1996). Several genetic risk factors have been identified for developing atherosclerosis, including hypertension, increased plasma levels of low density lipoprotein (LDL), decreased levels of high density lipoprotein, elevated lipoprotein (a), diabetes, and obesity (Glass and Witztum, *Cell,* 2001). In addition, smoking, lack of exercise, and a high fat diet are established environmental or behavioral risks linked to coronary artery disease and atherosclerosis.

The initiation and development of atherosclerotic lesions in the arterial wall proceeds in a series of well-defined stages. The first stage comprises the growth of fatty streak lesions in the sub-endothelial intimal space. Following the initial fatty streak formation, a fibrous plaque coalesces at the lesion site, harboring an acellular lipid core covered by a cap containing smooth muscle cells and extracellular matrix components. The final stage results in a complex lesion containing a necrotic core of macrophage and smooth muscle cell-derived foam cells along with deposits of extracellular cholesterol (Bennet, *Cardiovascular Res*, 1999; Davies, *Br Heart J,* 1993). The histological hallmark of emerging lesions is the

accumulation of macrophages and smooth muscle cells in the vascular wall intima, with the appearance of cholesteryl ester-rich foam cells (Brown, *J Cell Biol,* 1979; Ross, *Nature,* 1993). Prominent histological features accompanying lesion progression include intimal disorganization due to cell migration or proliferation and changes in the composition of the extracellular matrix. The fibrous cap and the thickening and deformation of the arterial wall characterize the advanced stages of lesion histopathology (Breslow, *Science*, 1996).

A variety of animal models have been developed to study the development of atherosclerosis (Breslow, *Science,* 1996; Bocan, *Curr Pharm Des*, 1998). These include rabbit models in which animals are fed a high cholesterol diet that leads to the formation of fatty streaks in the aorta, subsequent calcification and necrosis (Wojcicki, *Pol J Pharmacol Pharm* 1985) and the Watanabe strain (Tanzawa, *FEBS Lett,* 1980), which is genetically predisposed to develop atherosclerotic type lesions. Transgenic mouse models have been developed including mice deficient in the apolipoprotein E gene, a component of the lipoprotein particle that is a ligand for recognition and clearance by lipoprotein receptors (Zhang, *Science* 1992; Plump, *Cell*, 1992). These animals have delayed clearance of lipoproteins and subsequently elevated cholesterol, which leads to the development of atherosclerotic plaques. Another route of plasma lipid clearance from circulation involves recognition of lipoprotein particles by the LDL receptor. LDL-receptor deficient mice have been created that also have abnormally high levels of plasma cholesterol (Ishibashi, *Proc Natl Acad Sci* 1994; Ishibashi, *J Clin Invest,* 1993). Though this level is insufficient to create atherosclerotic lesions, feeding these animals a high cholesterol diet leads to massive lesion formation (Ishibashi, *J Clin Invest,* 1993; Breslow, *Science*, 1996).

An alternative rabbit model was developed where surgical perturbation in the periphery of the vascular wall leads to hyperplasia in the intima, closely resembling that seen in atherosclerotic lesions. This is accomplished by placing a soft silicone collar around the carotid arteries of rabbits (Martin, *Clin Exp Aller,* 2000; DeMeyer, *Arterioscler Thromb Vasc Biol,* 1997; Booth, *Atherosclerosis*, 1989). Within days, there is rapid proliferation of the smooth muscle cell component of the intima leading to vascular wall thickening and constriction of the vessel. This model has been used to attempt to identify factors controlling intimal cell proliferation and has implicated oxidated LDL (oxLDL) as a mediator of stimulation of smooth muscle cell growth (Matthys, *Arterioscler Thromb Vasc Biol,* 1997). Additional uses of this model have linked nitric oxide as a signaling factor inducing the extent of intimal thickening mediated by the collar (Marano, *Arterioscler Thromb Vasc Biol,* 1999; DeMeyer, *Arterioscler Thromb Vasc Biol,* 2000). This may be a component in

pathways leading to altered expression of other growth factors and matrix-degrading metalloproteinases which act together to remodel extracellular matrix and enhance cell migration and proliferation. However, these events do not seem to require a polymorphonuclear leukocyte population since blocking this type of cell infiltration has no effect on collar-induced intimal thickening (Van Put, *Arterioscler Thromb Vasc Biol,* 1998).

Development of these animal models has led to investigations into the possibility of direct pharmacological control of the pathological processes occurring in the arterial wall. Recent studies have focused on evaluating the direct effect of drug therapy on the cellular components of the arterial wall (Jackson, *Hypertension*, 1992). The anticipation is that by altering early events of the atherosclerotic process, the chances of halting or slowing the progression of the disease may be improved. Among the drugs under current investigation as anti-atherogenic agents are calcium channel blockers, or calcium antagonists, which are well established in the treatment of a number of cardiovascular disorders (Nayler, *Drugs*, 1993; Waters, *Am Heart J*, 1994). There are three subclasses of calcium channel antagonists: the phenylalkylamine derivatives (e.g. verapamil), the benzothiazepines (e.g., diltiazem), and the dihydropyridines (e.g., nifedipine, lercanidipine). All three subclasses modify calcium entry into cells by interacting with specific binding sites on the α_1 subunit of the L-type voltage-dependent calcium channel (Nayler, *Drugs*, 1993).

Calcium antagonists have been studied extensively in both *in vitro* and *in vivo* experimental models (Bernini, *Am J Cardiol*, 1989; Lichtor, *Appl Pathology*, 1989; Jackson, *Hypertension*, 1992; Henry, *Cardiovasc Pharm*, 1990; Catapano, *Eur Heart J,* 1997). In addition to evidence that calcium antagonists reduce blood pressure, experimental and clinical data indicate that calcium antagonists may protect against structural changes occurring in the vessel wall during the progression of atherosclerosis (Jackson, *Hypertension*, 1992; Nayler, *Biochem Pharmacol*, 1992; Lichtlen, *Cardiovasc Drugs Ther*, 1987; Parmley, *Am J Med*, 1987). Notably, several calcium-dependent processes contribute to atherogenesis, including lipid infiltration and oxidation, endothelial cell injury, chemotactic and growth factor activities, and smooth muscle cell migration and proliferation (Nayler, *Drugs*, 1993; Catapano, *Eur Heart J,* 1997).

Further, *in vivo* studies have shown that calcium antagonists protect against lesions induced by cholesterol feeding, endothelial injury, and experimental calcinosis (Bernini, *Am J Cardiol*, 1989; Keogh, *J Cardiovasc Pharmacol,* 1990; Weinstein, *Am J Med*, 1989; Catapano, *Ann N Y Acad Sci*, 1988). In addition, calcium antagonists have been shown to

decrease the accumulation of collagen, elastin, and proteoglycans in the arterial wall, following administration of compounds that induce atherosclerosis (Walters, *Circulation*, 1990).

Several *in vitro* models have supported these "anti-atherosclerotic" effects found *in vivo*. For instance, calcium antagonists have been shown to inhibit the migration and proliferation of smooth muscle cells *in vitro* (Nomoto, *Atherosclerosis*, 1988; Jackson, *Hypertension*, 1992; Corsini, *Pharmacol Res*, 1993; Bernini, *J Lipid Mediat Cell Signal*, 1994). In addition, calcium antagonists have been reported to modulate LDL cholesterol metabolism (Paoletti, *Ann N Y Acad Sci*, 1988) and to reduce fatty lesion development by interfering with cholesterol-esterification (Bernini, *J Hyperten Suppl*, 1993). Also, several studies have shown that calcium antagonists inhibit the uptake of lipids by macrophages (Daugherty, *Br J Pharm*, 1987; Bernini, *J Cardiovasc Pharm*, 1991; Schmitz, *Arteriosclerosis*, 1988; Stein, *Arteriosclerosis*, 1987).

A new dihydropyridine calcium antagonist, lercanidipine, has been shown to effectively reduce smooth muscle cell migration and proliferation *in vitro* (Corsini, *J Cardiovasc Pharm*, 1996). Lercanidipine has a high specificity for vascular smooth muscle cells and has a long duration of action due to its liposolubility. In addition, lercanidipine has been shown to modulate cholesterol acyl transferase activity and to act as an antioxidant for LDL in endothelial cell-mediated oxidation (Soma, *Br J Pharm*, 1998). Further *in vivo* studies have revealed that lercanidipine can inhibit both aortic fatty lesion deposition and carotid intimal hyperplasia (Soma, *Br J Pharm*, 1998).

Whether the effects of calcium antagonists on experimental atherosclerosis are linked to the blocking action on L-type channels remains unclear. Interestingly, lercanidipine presents with a chiral center that produces two enantiomers, of which the (R)-enantiomer is approximately 2-3 orders of magnitude less effective as a ligand to the calcium channel and in lowering blood pressure. Thus, studying the effects of the different enantiomers of lercanidipine provides a useful model for evaluating whether calcium antagonism plays a role in the anti-atherosclerotic activity of 1,4-dihydropyridine calcium antagonists.

In addition to the cited biochemical changes, atherosclerosis is also associated with altered gene expression that initiates cell proliferation and de-differentiation the intima of the arterial wall. The differential expression of genes during atherosclerosis development has been described in several cell types, including endothelial cells (Zhang, *Physiol Genomics*, 2001; de Waard, *Gene*, 1999), smooth muscle cells (Sobue, *Mol Cell Biochem*, 1999), and macrophages (Chiu, *Arterioscler Thromb Vasc Biol*, 1997; Krettek, *Arterioscler Thromb*

Vasc Biol, 1997). These reports suggest a complex transcriptional response initiated in cell types of the arterial wall during the development of the atherosclerotic phenotype.

Although the above studies have examined the differential expression of genes during early activation of arterial endothelial cells and have examined the expression of a few individual genes involved in the atherosclerotic phenotype, there has been no comprehensive study of the alteration of gene expression over time during the development of intimal hyperplasia in the rabbit carotid collar model. Nor has there been a study of the effects of calcium antagonists in gene expression during this type of lesion development. Thus, the number and identity of the genes that are differentially expressed during this type of cellular hypertrophy remains unknown. Further, the identity of those genes whose expression is affected by treatment with a calcium antagonist, such as lercanidipine, remains unknown.

The identification of genes whose level of expression is altered during the onset of atherosclerosis would not only contribute to the understanding of the disease pathology, but would also identify genes useful as diagnostic markers to indicate patients at risk for stroke or cardiovascular disease. Furthermore, the identification of differentially regulated genes would be useful to target genes for potential therapeutic intervention. In addition, the identification of genes whose expression is affected by calcium antagonists would advance the development of anti-atherosclerotic therapy that would target the specific action of calcium antagonists. The identification of such genes would also reveal key pathways that could be targeted for further investigation.

What is needed therefore, is an understanding and identification of the genes associated with atherosclerosis. The Total Gene Expression Analysis (TOGA™) method, described in Sutcliffe et al., Proc. Natl. Acad. Sci. USA 97(5): 1976-81 (2000), International published application WO 00/26406, U.S. Patent No. 5,459,037, U.S. Patent No. 5,807,680, U.S. Patent No. 6,030,784, U.S. Patent No. 6,096,503, U.S. Patent No. 6,110,680, and U.S. Patent No. 6,309,834, all of which are incorporated herein by reference, is a tool used to identify and analyze mRNA expression. The TOGA™ method is an improved method for the simultaneous sequence-specific identification of mRNAs in an mRNA population which allows the visualization of nearly every mRNA expressed by a tissue as a distinct band on a gel whose intensity corresponds roughly to the concentration of the mRNA. The method can identify changes in expression of mRNA associated with the administration of drugs or with physiological or pathological conditions such as atherosclerosis.

SUMMARY OF THE INVENTION

The present invention associates identified polynucleotides and their encoded polypeptides to atherosclerosis such that the polynucleotides and polypeptides may be useful for diagnosis and treatment of atherosclerosis. One embodiment of the invention provides a method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject. The method comprises determining the presence or absence of a mutation in a polynucleotide of the invention. A pathological condition or a susceptibility to a pathological condition, such as atherosclerosis is diagnosed based on the presence or absence of the mutation.

Even another embodiment of the invention provides a method of diagnosing a pathological condition or a susceptibility to a pathological condition, such as atherosclerosis, in a subject. Especially preferred embodiments include methods of diagnosing atherosclerosis. The method comprises detecting an alteration in expression of a polypeptide encoded by the polynucleotide of the invention, wherein the presence of an alteration in expression of the polypeptide is indicative of the pathological condition or susceptibility to the pathological condition. The alteration in expression can be an increase in the amount of expression or a decrease in the amount of expression. In a preferred embodiment a first biological sample is obtained from a patient suspected of having atherosclerosis and a second sample from a suitable comparable control source is obtained. The amount of at least one polypeptide encoded by a polynucleotide of the invention is determined in the first and second sample. The amount of the polypeptide in the first and second samples is determined. A patient is diagnosed as having atherosclerosis if the amount of the polypeptide in the first sample is greater than or less than the amount of the polypeptide in the second sample.

Where a polynucleotide of the invention is down-regulated and exacerbates a pathological condition, such as atherosclerosis, the expression of the polynucleotide can be increased or the level of the intact polypeptide product can be increased in order to treat, prevent, ameliorate, or modulate the pathological condition. This can be accomplished by, for example, administering a polynucleotide or polypeptide of the invention to the mammalian subject.

Where a polynucleotide of the invention is up-regulated and exacerbates a pathological condition in a mammalian subject, such as atherosclerosis, the expression of the polynucleotide can be blocked or reduced or the level of the intact polypeptide product can be reduced in order to treat, prevent, ameliorate, or modulate the pathological condition. This can be accomplished by, for example, the use of antisense oligonucleotides, triple helix base

pairing methodology or ribozymes. Alternatively, drugs or antibodies that bind to and inactivate the polypeptide product can be used.

Additionally, the present invention provides novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant methods for producing the polynucleotides and the polypeptides. One embodiment of the invention provides an isolated nucleic acid molecule comprising a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. Also provided is an isolated nucleic acid molecule comprising a polynucleotide at least 95% identical to any one of these isolated nucleic acid molecules and an isolated nucleic acid molecule at least ten bases in length that is hybridizable to any one of these isolated nucleic acid molecules under stringent conditions. Any one of these isolated nucleic acid molecules can comprise sequential nucleotide deletions from either the 5'-terminus or the 3'-terminus. Further provided is a recombinant vector comprising any one of these isolated nucleic acid molecules and a recombinant host cell comprising any one of these isolated nucleic acid molecules. Also provided is the gene corresponding to the cDNA sequence of any one of these isolated nucleic acids.

Another embodiment of the invention provides an isolated polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. Also provided is an isolated nucleic acid molecule encoding any of these polypeptides, an isolated nucleic acid molecule encoding a fragment of any of these polypeptides, an isolated nucleic acid molecule encoding a polypeptide epitope of any of these polypeptides, and an isolated nucleic acid encoding a species homologue of any of these polypeptides. Preferably, any one of these polypeptides has biological activity. Optionally, any one of the isolated polypeptides comprises sequential amino acid deletions from either the C-terminus or the N-terminus. Further provided is a recombinant host cell that expresses any one of these isolated polypeptides.

Yet another embodiment of the invention comprises an isolated antibody that binds specifically to an isolated polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID

NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. The isolated antibody can be a monoclonal antibody or a polyclonal antibody.

Another embodiment of the invention provides a method for preventing, treating, modulating, or ameliorating a medical condition, such as atherosclerosis, comprising administering to a mammalian subject a therapeutically effective amount of a polypeptide of the invention or a polynucleotide of the invention.

A further embodiment of the invention provides an isolated antibody that binds specifically to the isolated polypeptide of the invention. A preferred embodiment of the invention provides a method for preventing, treating, modulating, or ameliorating a medical condition, such as atherosclerosis, comprising administering to a mammalian subject a therapeutically effective amount of the antibody. In one preferred embodiment, a method for preventing, treating, modulating or ameliorating atherosclerosis, is provided.

Another embodiment of the invention provides a method for identifying a binding partner to a polypeptide of the invention. A polypeptide of the invention is contacted with a binding partner and it is determined whether the binding partner effects an activity of the polypeptide.

Yet another embodiment of the invention is a method of identifying an activity of an expressed polypeptide in a biological assay. A polypeptide of the invention is expressed in a cell and isolated. The expressed polypeptide is tested for an activity in a biological assay and the activity of the expressed polypeptide is identified based on the test results.

Still another embodiment of the invention provides a substantially pure isolated DNA molecule suitable for use as a probe for genes regulated in atherosclerosis, chosen from the group consisting of the DNA molecules shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

Even another embodiment of the invention provides a kit for detecting the presence of a polypeptide of the invention in a mammalian tissue sample. The kit comprises a first antibody which immunoreacts with a mammalian protein encoded by a gene corresponding to the polynucleotide of the invention or with a polypeptide encoded by the polynucleotide in an amount sufficient for at least one assay and suitable packaging material. The kit can further comprise a second antibody that binds to the first antibody. The second antibody can be

labeled with enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, or bioluminescent compounds.

Another embodiment of the invention provides a kit for detecting the presence of genes encoding a protein comprising a polynucleotide of the invention, or fragment thereof having at least 10 contiguous bases, in an amount sufficient for at least one assay, and suitable packaging material.

Yet another embodiment of the invention provides a method for detecting the presence of a nucleic acid encoding a protein in a mammalian tissue sample. A polynucleotide of the invention or fragment thereof having at least 10 contiguous bases is hybridized with the nucleic acid of the sample. The presence of the hybridization product is detected.

Another embodiment of the invention provides a method of diagnosing or monitoring the effects of treating a subject with a dihydropyridine calcium antagonist comprising detecting an alteration in expression of at least one polypeptide encoded by at least one polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. The dihydropyridine calcium antagonist is lercanidipine.

Yet another embodiment of the invention provides a method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist used to control gene expression. The dihydropyridine calcium channel antagonist is lercanidipine.

Another embodiment of the invention provides a method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that decreases expression of the polynucleotide of SEQ ID NO:9 and SEQ ID NO:11 and increases the expression of the polynucleotide of SEQ ID NO:13.

Still another embodiment of the invention provides a method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that decreases the expression of the polypeptide encoded by the polynucleotide of SEQ ID

NO:9 and SEQ ID NO:11 and increases the expression of the polypeptide encoded by the polynucleotide of SEQ ID NO:13.

Yet another embodiment of the invention provides a method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that decreases the expression of the polynucleotide of SEQ ID NO:9 and SEQ ID NO:11.

Another embodiment of the invention provides a method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that decreases the expression of the polypeptide encoded by the polynucleotide of SEQ ID NO:9 and SEQ ID NO:11.

Still another embodiment of the invention provides a method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that increases the expression of the polynucleotide of SEQ ID NO:13.

Another embodiment of the invention provides a method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that increases the expression of the polypeptide encoded by the polynucleotide of SEQ ID NO:13. The medical condition is atherosclerosis, and the dihydropyridine calcium channel antagonist is lercanidipine.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description, appended claims, and accompanying drawings where:

Figure 1 is a graphical representation of the results of TOGA™ runs using a 5' PCR primer with parsing bases GACC (SEQ ID NO:28) and the universal 3' PCR primer (SEQ ID NO:21) showing PCR products produced from mRNA extracted from rabbit carotids in animals under the following conditions: carotid collar-implanted and treated with vehicle (1 day) (Panel A), carotid collar-implanted and treated with lercanidipine (1day) (Panel B), sham-operated and treated with vehicle (1 day) (Panel C), sham-operated and treated with lercanidipine (1 day) (Panel D), carotid collar-implanted and treated with vehicle (7 days) (Panel E), sham-operated and treated with vehicle (7 days) (Panel F), carotid collar-implanted

and treated with lercanidipine (7 days) (Panel G), and sham-operated and treated with lercanidipine (7 days) (Panel H), where the vertical index line indicates a PCR product of about 456 base pairs (b.p.). The horizontal axis represents the number of base pairs of the molecules in these samples and the vertical axis represents the fluorescence measurement in the TOGA™ analysis (which corresponds to the relative expression of the molecule of that address). The data presented in panels E, F, and H are plotted for independent, duplicate runs for these three RNA samples. The TOGA™ data indicate that the relative expression of this molecule is greatest in the sham-operated 7 day samples (Panel F & H) compared to collar-implanted day 7 samples or to the sham-operated 1 day samples.

The results of the TOGA™ runs have been normalized using the methods described in pending U.S. Patent Application Serial No. 09/318,699/U.S., and pending PCT Application Serial No. PCT/US00/14159, both entitled Methods and System for Amplitude Normalization and Selection of Data Peaks (Dennis Grace, Jayson Durham); and pending U.S. Patent Application Serial No. 09/318,679/U.S. and pending PCT Application Serial No. PCT/US00/14123, both entitled Methods for Normalization of Experimental Data (Dennis Grace, Jayson Durham) all of which are incorporated herein by reference. The vertical line drawn through the 8 panels represents the DST molecule identified as REC2_7 (SEQ ID NO:7).

Figure 2 presents a graphical example of the results obtained when a DST is verified by the Extended TOGA™ method using a primer generated from a direct sequenced product (as described below). The length of the PCR product corresponding to SEQ ID NO:7 (DST REC2_7) was direct sequenced and a 5' PCR primer was built from the direct sequenced DST (SEQ ID NO:29). The product obtained from PCR with this primer (SEQ ID NO:29) and the universal 3' PCR primer (SEQ ID NO:21) (as shown in the top panel) was compared to the length of the original PCR product that was produced in the TOGA™ reaction with mRNA extracted from the sham-operated and lercanidipine-treated day 7 sample using a 5' PCR primer with parsing bases GACC (SEQ ID NO:28) and the universal 3' PCR primer (SEQ ID NO:21) (as shown in the middle panel). Again, for all panels, the number of base pairs is shown on the horizontal axis, and fluorescence intensity (which corresponds to relative expression) is found on the vertical axis. In the bottom panel, the traces from the top and middle panels are overlaid, demonstrating that the peak found using an extended primer from the direct sequenced DST is the same number of base pairs as the original PCR product obtained through TOGA™ as DST REC2_7 (SEQ ID NO:7).

Figure 3 is a graphical representation of the results of TOGA™ for 4 separate DSTs using 5' PCR primers with parsing bases AAAC299 (SEQ ID NO:30) (Figure 3 Panel A), GCGT197 (SEQ ID NO:31) (Figure 3 Panel B), TCGG366 (SEQ ID NO:32) (Figure 3 Panel C), and TCAA450 (SEQ ID NO:33) (Figure 3 Panel D) obtained from separate PCR reactions in combination with the universal 3' PCR primer (SEQ ID NO:21). The resulting PCR products were produced from mRNA extracted from rabbit carotids in animals under the following conditions: carotid collar-implanted and treated with vehicle (1 day) (Con Collar 1), carotid collar-implanted and treated with lercanidipine (1day) (Ler Collar 1), sham-operated and treated with vehicle (1 day) (Con Sham 1), sham-operated and treated with lercanidipine (1 day) (Ler Sham 1), carotid collar-implanted and treated with vehicle (7 days) (Con Collar 7), sham-operated and treated with vehicle (7days) (Con Sham 7), carotid collar-implanted and treated with lercanidipine (7 days) (Ler Collar 7), and sham-operated and treated with lercanidipine (7 days) (Ler Sham 7). The vertical line drawn through the 8 panels represents the DST molecule identified as REC2_1 (SEQ ID NO:1) for Figure 3 Panel A, REC2_9 (SEQ ID NO:9) for Figure 3 Panel B, REC2_14 (SEQ ID NO:14) for Figure 3 Panel C, and REC2_13 (SEQ ID NO:13) for Figure 3 Panel D. The results of the TOGA™ runs have been normalized using the methods as described above for Figure 1. The data shown in Figure 3 Panel A for AAAC299 indicate a DST whose expression is greatest in the collar-implanted animals (day 7 samples) compared to sham-operated animals (day 7 samples) and is expressed at low levels in all day 1 samples. The data shown in Figure 3 Panel B for GCGT197 illustrate a DST whose expression is increased by the presence of the collar in the vehicle-treated animals (day 7 samples) compared to sham operated animals (day 7 samples) but not in the presence of the collar in the lercanidipine-treated animals (day 7 samples) compared to sham operated, lercanidipine-treated animals (day 7 samples) and is expressed at low levels in all day 1 samples. The data shown in Figure 3 Panel C for TCGG366 indicate a DST whose expression is decreased by the presence of the collar in the vehicle-treated or lercanidipine-treated animals (day 7 samples) compared to sham-operated animals (day 7 samples). The data shown in Figure 3 Panel D for TCAA450 indicate a DST whose expression is decreased by the presence of the collar in the vehicle-treated animals (day 7 samples) compared to sham-operated animals (day 7 samples) but not in the presence of the collar in the lercanidipine-treated animals (day 7 samples) compared to sham-operated, lercanidipine-treated animals (day 7 samples).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention and the methods of obtaining and using the present invention will be described in detail after setting forth some preliminary definitions.

Definitions

The following definitions are provided to facilitate understanding of certain terms used in the present invention. Many of the techniques described herein are described in Dracopoli, *Current Protocols in Human Genetics*, 1994, and Ausubel, *Current Protocols in Molecular Biology*, 2000, both of which are incorporated herein by reference.

An "**isolated nucleic acid**" refers to a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid or to that of any fragment of naturally occurring genomic nucleic acid. The term therefore covers, for example, (a) a DNA which has the sequence of part of a naturally occurring genomic DNA molecule but is not flanked by both of the coding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein; and (e) a nucleic acid synthesized through chemical means.

An "**isolated polypeptide**" refers to a polypeptide removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state.

An "**isolated antibody**" refers to an antibody removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state.

"**Isolated**" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state.

"**Polynucleotide**" or "**polynucleotide of the invention**" or "**polynucleotide of the present invention**" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID

NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. For example, the polynucleotide can contain all or part of the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. A polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, a polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms. A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 or the complement thereof, or the cDNA.

"**Stringent hybridization conditions**" refers to an overnight incubation at 42°C in a solution comprising 50% formamide, 5X SSC (5X SSC = 750 mM NaCl, 75 mM sodium citrate, 50 mM sodium phosphate pH 7.6), 5X Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1X SSC at about 65°C. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH_2PO_4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50°C

with 1X SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC). Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO (5% w/v non-fat dried milk in phosphate buffered saline ("PBS"), heparin, denatured salmon sperm DNA, and other commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility. Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

"**Polypeptide**" or "**polypeptide of the invention**" or "**polypeptide of the present invention**" refers to a molecule having a translated amino acid sequence generated from the polynucleotide as broadly defined. The translated amino acid sequence, beginning with the methionine, is identified although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by the translation of these alternative open reading frames are specifically contemplated by the present invention. The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. See references below. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation,

covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formulation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, e.g., Creighton, *Proteins – Structure And Molecular Properties*, 2nd Ed., 1993; Johnson, *Posttranslational Covalent Modification Of Proteins*, 1983; Seifter, *Meth Enzymol*, 1990; Rattan, *Ann N Y Acad Sci*, 1992).

A polypeptide has "**biological activity**" when the polypeptide has structural, regulatory or biochemical functions of a naturally occurring molecule. Biological activity can be measured by several kinds of biological assays, both in vitro (e.g., cell cultures) or in vivo (e.g., behavioral or metabolic assays). In these cases, the potency of the biological activity is measured by its dose-response characteristics; in the case of polypeptides with activity similar to the polypeptide of the present invention, the dose-response dependency will be substantially similar in a given activity as compared to the polypeptide of the present invention. Polypeptides may derive their "biological activity" through binding to specific cellular receptors, which mediate secondary signals to the target cell or tissue. In other cases, they may have direct effects in the absence of receptor mediated binding or signaling. For example, peptides may interact directly with other proteins or other molecules, and alter their conformation of function, or they may block the binding of a third molecule to the same interaction site, thereby affecting the signal normally mediated between the two molecules.

"**DNA**" refers to deoxyribonucleic acid.

"**RNA**" refers to ribonucleic acid.

"**mRNA**" refers to messenger ribonucleic acid.

"**cDNA**" refers to a deoxyribonucleic acid that is complementary to an mRNA.

Gene" refers to a region of DNA that controls a discrete hereditary characteristic, usually corresponding to a single protein or RNA. This definition includes the entire functional unit encompassing coding DNA sequence, the regions preceding and following the coding region (leader or trailer), noncoding regulatory DNA sequences, and introns.

"**Codon**" refers to the three-nucleotide sequence of an mRNA molecule that codes for one specific amino acid.

"**Vector**" refers to a vehicle for transfer of DNA into a recipient cell.

"**Silent mutation**" or "silent substitution" refers to a mutation that causes no functional change in the gene product.

"**Phenotype**" refers to the appearance, behavior, or other characteristics of a cell or individual due to actual expression, or pattern of expression, of a specific gene or set of genes. Differences in phenotype may be due to changes in the expression or pattern of expression of a specific gene or set of genes, or to differences in the biological activity of one or more genes. These differences may be a result of polymorphic or allelic differences in the coding region of the specific genes or in their regulatory sequences, or to other genetic variations (e.g., new mutations).

"**Hybridization**" refers to the time- and temperature-dependent process by which two complementary single-stranded polynucleotides associate to form a double helix.

"**Probe**" refers to a polynucleotide, often radiolabelled, used to detect complementary sequences, e.g., an mRNA used to locate its gene by a corresponding nucleic acid blotting method.

"**Conservative amino acid substitution**" refers to a substitution between similar amino acids that preserves an essential chemical characteristic of the original polypeptide.

"**Phage**" refers to a virus that infects bacteria. Many phage have proved useful in the study of molecular biology and as vectors for the transfer of genetic information between cells.

"**Plasmid**" refers to a self-replicating extra-chromosomal element, usually a small segment of duplex DNA that occurs in some bacteria; used as a vector for the introduction of new genes into bacteria.

"**Retrovirus**" refers to a virus with an RNA genome that may be either an mRNA, (+)-RNA, or its complement, (-)-RNA. Class 1 contains (+)-RNA; class 2, (-)-RNA, which is the template for an RNA-dependent RNA polymerase; class 3, double-stranded RNA, in which (+)-RNA is synthesized by an RNA-dependent RNA polymerase; class 4, retrovirus, in which (+)-RNA is a template for an RNA-dependent DNA polymerase (a reverse transcriptase). A Retrovirus may be used as a vector for the introduction of genes into mammalian cells.

"**Triple Helix**" refers to the tertiary structure of collagen that twists three polypeptide chains around themselves; also a triple-stranded DNA structure that involves Hoogstein base pairing between B-DNA and a third DNA strand that occupies the major groove.

"**Antibody**" refers to an immunoglobulin molecule that reacts specifically with another (usually foreign) molecule, the antigen.

"**Monoclonal antibody (mAb)**" refers to an immunoglobulin preparation that is completely homogeneous, due to its formation by daughters of a single progenitor cell that has been programmed for the synthesis and secretion of one specific antibody.

"**Polyclonal antibody**" refers to a heterogeneous immunoglobulin preparation that contains antibodies directed against one or more determinants on an antigen; the product of daughters of several progenitor cells that have been programmed for immunoglobulin synthesis and secretion.

"**Complementary**" as used in nucleic acid chemistry, is descriptive of the relationship between two polynucleotides that can combine in an antiparallel double helix; the bases of each polynucleotide are in a hydrogen-bonded inter-strand pair with a complementary base, A to T (or U) and C to G. In protein chemistry, the matching of shape and/or charge of a protein to a ligand.

"**C-terminus**" refers to, in a polypeptide, the end with a free carboxyl group.

"**N-terminus**" refers to, in a polypeptide, the end with a free amino group.

A "**secreted**" protein refers to those proteins capable of being directed to the endoplasmic reticulum, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

"**Variant**" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. In general, variants have close similarity overall and are identical in many regions to the polynucleotide or polypeptide of the present invention.

"**Identity**" per se has an art-recognized meaning and can be calculated using published techniques. (See, e.g., Lesk, *Computational Molecular Biology. Sources and Methods for Sequence Analysis*, 1988; Smith, *Biocomputing: Informatics and Genome Projects*, 1993; Griffin, *Computer Analysis Of Sequence Data, Part I*, 1994; von Heinje, *Sequence Analysis In Molecular Biology*, 1987; and Gribskov, *Sequence Analysis Primer*, 1991). While there exists a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans

(Carillo, *SIAM J Applied Math*, 1988). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Bishop, *Guide to Huge Computers*, 1994, and Carillo, *SIAM J Applied Math*, 1988.

"**Epitopes**" refer to polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to a polypeptide fragment comprising an epitope, as well as the polynucleotide encoding this fragment. A region of a protein molecule to which an antibody can bind is defined as an "**antigenic epitope**." In contrast, an "**immunogenic epitope**" is defined as a part of a protein that elicits an antibody response. (See, e.g., Geysen, *Proc Natl Acad Sci,* 1984).

"**Homologous**" means corresponding in structure, position, origin or function.

A "**homologous polynucleotide**" refers to a polynucleotide which encodes a homologous polypeptide.

A "**homologous nucleic acid molecule**" refers to a nucleic acid molecule which encodes a homologous polypeptide.

A "**homologous polypeptide**" refers to a polypeptide having any of the following characteristics with respect to the polypeptides of the present invention: similar function, similar amino acid sequence, similar subunit structure and formation of a functional heteropolymer, immunological cross-reaction, similar expression profile, similar subcellular location, similar substrate specificity, or similar response to specific inhibitors.

"**ELISA**" refers to an enzyme-linked immunosorbent assay that employs an antibody or antigen bound to a solid phase and an enzyme-antigen or enzyme-antibody conjugate to detect and quantify the amount of an antigen present in a sample.

A "**specific binding agent**" refers to a molecular entity capable of selectively binding a reagent species of the present invention or a complex containing such a species, but is not itself a polypeptide or antibody molecule composition of the present invention.

The word "**complex**" as used herein refers to the product of a specific binding reaction such as an antibody-antigen or receptor-ligand reaction. Exemplary complexes are immunoreaction products.

As used herein, the terms "**label**" and "**indicating means**" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal to indicate the presence of a complex.

The term "**package**" refers to a solid matrix or material such as glass, plastic (e.g., polyethylene, polypropylene, or polycarbonate), paper, foil and the like capable of holding within fixed limits a polypeptide, polyclonal antibody, or monoclonal antibody of the present

invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated polypeptide or antibody or it can be a microtiter plate well to which microgram quantities of a contemplated polypeptide or antibody have been operatively affixed (i.e., linked) so as to be capable of being immunologically bound by an antibody or antigen, respectively.

"**Instructions for use**" typically include a tangible expression describing the reagent concentration or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

"**DST**" refers to a Digital Sequence Tag, i.e., a polynucleotide that is an expressed sequence tag of the 3' end of an mRNA.

Other terms used in the fields of biotechnology and molecular and cell biology as used herein will be as generally understood by one of ordinary skill in the applicable arts.

Background

These experiments are intended to illustrate the invention, and are not to be construed as limiting the scope of the invention.

Studies were designed to identify carotid transcripts that are regulated by proliferative lesions induced by application of a silastic carotid collar and also to identify carotid transcripts responsive to lercanidipine treatment. The TOGA™ (TOtal Gene expression Analysis) method was used to identify digital sequence tags (DSTs) corresponding to mRNAs whose expression is regulated by proliferative lesion development caused by mechanically induced intimal hyperplasia, regulated by lercanidipine treatment, or regulated by proliferative lesions and reversed by lercanidipine treatment.

New Zealand male rabbits weighing 2.3-2.8 kg (Charles River, Calco, Italy) were housed with free access to standard diet and water and maintained on 12 hr forced light-dark cycle at 22-24° C during the experiments. The rabbits were divided into four groups:

(1) control group (n=80); rabbits treated with vehicle + carotid collar for 1 day.

(2) lercanidipine group (n=80); rabbits treated with lercanidipine + carotid collar for 1 day.

(3) control group (n=80); rabbits treated with vehicle + carotid collar for 7 days.

(4) lercanidipine group (n=80); rabbits treated with lercanidipine + carotid collar for 7 days.

The rabbits were implanted with carotid silastic collar in order to induce proliferative lesions in the right carotid artery and the contralateral artery was treated as sham-operated vessel. At different times after collar implantation, animals treated with vehicle or with lercanidipine were sacrificed, both carotids were carefully removed and RNA was immediately extracted and purified.

Each animal produced a "collared" carotid and a sham-operated carotid to obtain a total of 8 different samples of carotid RNA, as shown below.

Group	Treatment	Day	RNA Sample
Rabbits group 1	vehicle + carotid collar	Day 1	RNA sample (1)
	vehicle + sham-operated	Day 1	RNA sample (2)
Rabbits group 2	lercanidipine + carotid collar	Day 1	RNA sample (3)
	lercanidipine + sham-operated	Day 1	RNA sample (4)
Rabbits group 3	vehicle + carotid collar	Day 7	RNA sample (5)
	vehicle + sham-operated	Day 7	RNA sample (6)
Rabbits group 4	lercanidipine + carotid collar	Day 7	RNA sample (7)
	lercanidipine + sham-operated	Day 7	RNA sample (8)

For the induction of carotid lesions, animals were anesthetized by intramuscular injection of 5 mg/kg xylazine (Rompun, Bayer AG, Leverkusen, Germany) and 35 mg/kg Ketamine (Inoketam, Virbac, Milano, Italy), using sterile disposable syringes (Terumo Europe, N.V. 3001 Leuven, Belgium). After 20 minutes, rabbits were placed in dorsal recumbency and sheared at the level of the trachea with an Aesculap Favorita II small animal clipper, (Aesculap AG, 78532 Tuttlingen, Germany). A midline longitudinal neck incision was made with a sterile scalpel (Maersk Medical Ltd., England), and both carotid arteries were surgically exposed by separating them from surrounding tissues and vagal nerve fibre connections. A nonocclusive, biologically inert, soft, hollow Silastic collar (Silicollar, MediGene Oy, Kuopio, Finland) was positioned around both carotid arteries by gentle manipulation of the vessel. The collar was 2 cm in length and it touched the artery circumference at two points 1.5 cm apart. Right carotids (injured artery, positive control) maintained the collar for all the duration of the study, until sacrifice. In the left carotid (sham-operated artery) the collar was removed just before tissues were reseated anatomically

and the wounds sutured with B204/14 suture needles (Acufirm) and 3/0 sterile silk surgical thread (Ethicon GmbH & Co. KG, Germany). At the end of experimental period animals were killed with a lethal dose of urethane (25% w/v in 0.1 M phosphate buffered saline (PBS), 1 ml/250g body weight) (Sigma, Milano, Italy) injected in the marginal ear vein by the use of disposable pyrogen free and sterile infusion set 25Gx3/4", 0.50X19 mm, 0.46 ml Mirage (Artsana, Como, Italy). Immediately after euthanasia the collared portion of the right carotids and the corresponding segments of the controlateral sham-operated arteries were readily dissected and excised. Tissues were then rapidly washed in sterile PBS, treated with diethyl pyrocarbonate (DEPC) (Sigma, Milano, Italy) and chilled on ice. Total RNA was immediately purified.

The hydrochloride salt of lercanidipine (Recordati, Milano, Italy) was administered subcutaneously as solution in 50% propylene glycol. The dose of lercanidipine utilized (3 mg/kg every three days, subcutaneously) did not affect arterial blood pressure. Lercanidipine was administered at days -7, -4, -1, +2, +5 considering the carotid collar implant day as day 0. Vehicle treated animals were managed in the same conditions.

Carotid mRNAs were prepared as follows. First, the carotids from each experimental group were retrieved and shredded using a polytron homogenizer. The nuclei and blood cells were pelleted by centrifugation. The supernatants were extracted twice with phenol-chloroform-isoamyl alcohol and once with chloroform-isoamyl alcohol. Total RNA was then precipitated from the aqueous phase with ethanol. The polyA+ mRNA was prepared using standard methods of polyA selection known in the art (Schibler, *J Mol Biol* 1980).

The TOGA™ Process

Isolated RNA was analyzed using a method of simultaneous sequence-specific identification of mRNAs known as TOGA™ (TOtal Gene expression Analysis) described in Sutcliffe, *Proc Natl Acad Sci*, 2000; International published application WO 00/26406; U.S. Patent No. 5,459,037; U.S. Patent No. 5,807,680; U.S. Patent No. 6,030,784; U.S. Patent No. 6,096,503, U.S. Patent 6,110,680, and U.S. Patent 6,309,834, hereby incorporated herein by reference. Preferably, prior to the application of the TOGA™ technique, the isolated RNA was enriched to form a starting polyA-containing mRNA population by methods known in the art. In a preferred embodiment, the TOGA™ method further comprised an additional PCR step performed using four 5' PCR primers in four separate reactions and cDNA templates prepared from a population of antisense cRNAs. A final PCR step that used 256 5' PCR primers in separate reactions produced PCR products that were cDNA fragments that corresponded to the 3'-region of the starting mRNA population. The produced PCR products

were then identified by: a) the initial 5' sequence comprising the sequence remainder of the recognition site of the restriction endonuclease used to cut and isolate the 3' region plus the sequence of the preferably four parsing bases immediately 3' to the remainder of the recognition site, preferably the sequence of the entire fragment, and b) the length of the fragment. These two parameters, sequence and fragment length, were used to compare the obtained PCR products to a database of known polynucleotide sequences. Since the length of the obtained PCR products includes known vector sequences at the 5' and 3' ends of the insert, the sequence of the insert provided in the sequence listing is shorter than the fragment length that forms part of the digital address.

The method yields Digital Sequence Tags (DSTs), that is, polynucleotides that are expressed sequence tags of the 3' end of mRNAs. DSTs that showed changes in relative levels during lercanidipine treatment or in response to carotid collar implantation were selected for further study. The intensities of the laser-induced fluorescence of the labeled PCR products were compared across samples isolated.

In general, double-stranded cDNA is generated from poly(A)-enriched cytoplasmic RNA extracted from the tissue samples of interest using an equimolar mixture or set of all 48 5'-biotinylated anchor primers to initiate reverse transcription. One such suitable set is G-A-A-T-T-C-A-A-C-T-G-G-A-A-G-C-G-G-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N-N (SEQ ID NO: 18), where V is A, C or G and N is A, C, G or T. One member of this mixture of 48 anchor primers initiates synthesis at a fixed position at the 3' end of all copies of each mRNA species in the sample, thereby defining a 3' endpoint for each species, resulting in biotinylated double-stranded cDNA.

Each biotinylated double-stranded cDNA sample was cleaved with the restriction endonuclease MspI, which recognizes the sequence CCGG. The resulting fragments of cDNA corresponding to the 3' region of the starting mRNA were then isolated by capture of the biotinylated cDNA fragments on a streptavidin-coated substrate. Suitable streptavidin-coated substrates include microtitre plates, PCR tubes, polystyrene beads, paramagnetic polymer beads, and paramagnetic porous glass particles. A preferred streptavidin-coated substrate is a suspension of paramagnetic polymer beads (Dynal, Inc., Great Neck, NY).

After washing the streptavidin-coated substrate and captured biotinylated cDNA fragments, the cDNA fragment product was released by digestion with NotI, which cleaves at an 8-nucleotide sequence within the anchor primers but rarely within the mRNA-derived portion of the cDNAs. The 3' MspI-NotI fragments, which are of uniform length for each mRNA species, were directionally ligated into ClaI- NotI-cleaved plasmid pBC SK+

(Stratagene, La Jolla, CA) in an antisense orientation with respect to the vector's T3 promoter, and the product used to transform Escherichia coli SURE cells (Stratagene). The ligation regenerates the NotI site, but not the MspI site, leaving CGG as the first 3 bases of the 5' end of all PCR products obtained. Each library contained in excess of 5×10^5 recombinants to ensure a high likelihood that the 3' ends of all mRNAs with concentrations of 0.001% or greater were multiply represented. Plasmid preps (Qiagen) were made from the cDNA library of each sample under study.

An aliquot of each library was digested with MspI, which effects linearization by cleavage at several sites within the parent vector while leaving the 3' cDNA inserts and their flanking sequences, including the T3 promoter, intact. The product was incubated with T3 RNA polymerase (MEGAscript kit, Ambion) to generate antisense cRNA transcripts of the cloned inserts containing known vector sequences abutting the MspI and NotI sites from the original cDNAs.

At this stage, each of the cRNA preparations was processed in a three-step fashion. In step one, 250 ng of cRNA was converted to first-strand cDNA using the 5' RT primer (A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G, (SEQ ID NO: 19). In step two, 400 pg of cDNA product was used as PCR template in four separate reactions with each of the four 5' PCR primers of the form G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N (SEQ ID NO: 20), each paired with a "universal" 3' PCR primer G-A-G-C-T-C-C-A-C-C-G-C-G-G-T (SEQ ID NO: 21) to yield four sets of PCR reaction products ("N1 reaction products").

In step three, the product of each subpool was further divided into 64 subsubpools (2ng in 20µl) for the second PCR reaction. This PCR reaction comprised adding 100 ng of the fluoresceinated "universal" 3' PCR primer (SEQ ID NO: 21) conjugated to 6-FAM and 100 ng of the appropriate 5' PCR primer of the form C-G-A-C-G-G-T-A-T-C-G-G-N-N-N-N (SEQ ID NO: 22), and using a program that included an annealing step at a temperature X slightly above the Tm of each 5' PCR primer to minimize artifactual mispriming and promote high fidelity copying. Each polymerase chain reaction step was performed in the presence of TaqStart antibody (Clonetech).

The products ("N4 reaction products") from the final polymerase chain reaction step for each of the tissue samples were resolved on a series of denaturing DNA sequencing gels using the automated ABI Prizm 377 sequencer. Data were collected using the GeneScan software package (ABI) and normalized for amplitude and migration. Complete execution of this series of reactions generated 64 product subpools for each of the four pools established

by the 5' PCR primers of the first PCR reaction, for a total of 256 product subpools for the entire 5' PCR primer set of the second PCR reaction.

Identifying genes regulated in the carotid artery in response to carotid collar implantation and lercanidipine

The mRNA samples extracted from rabbit carotids in carotid collar-implanted animals treated with vehicle (1 day), carotid collar-implanted animals treated with lercanidipine (1day), sham-operated animals treated with vehicle (1 day), sham-operated animals treated with lercanidipine (1 day), carotid collar-implanted animals treated with vehicle (7 days), sham-operated animals treated with vehicle (7 days), carotid collar-implanted animals treated with lercanidipine (7 days), and sham-operated animals treated with lercanidipine (7 days) as described above were analyzed. Table 1 is a summary of the expression levels of 50 mRNAs determined from cDNA. These cDNA molecules are identified by their digital address, that is, a partial 5' terminus nucleotide sequence coupled with the length of the molecule, as well as the relative amount of the molecule produced at different time intervals after treatment. The 5' terminus partial nucleotide sequence is determined by the recognition site for MspI (CC GG) and the nucleotide sequence of the parsing bases of the 5' PCR primer used in the final PCR step. The digital address length of the fragment was determined by interpolation on a standard curve and, as such, may vary ± 1-2 b.p. from the actual length as determined by sequencing.

For example, the entry in Table 1 that describes a DNA molecule identified by the digital address MspI GACC, is further characterized as having a 5' terminus partial nucleotide sequence of CGGGACC and a digital address length of 456 b.p. The DNA molecule identified as MspI GACC456 is further described as being expressed at greatest levels in the sham-operated 7 day samples (vehicle and lercanidipine samples) compared to collar-implanted day 7 samples (vehicle and lercanidipine samples) or to the sham-operated 1 day samples (vehicle and lercanidipine samples) (see Figure 1). Additionally, the DNA molecule identified as MspI GACC456 is described by its nucleotide sequence, which corresponds with SEQ ID NO: 7.

Similarly, the other DNA molecules identified in Table 1 by their MspI digital addresses are further characterized by: 1) the level of gene expression in the carotid collar-implanted, vehicle-treated control day 1 sample, 2) the level of gene expression in the carotid collar-implanted, lercanidipine-treated day 1 sample, 3) the level of gene expression in the sham-operated, vehicle-treated control day 1 sample, 4) the level of gene expression in the

sham-operated, lercanidipine-treated day 1 sample, 5) the level of gene expression in the carotid collar-implanted, vehicle-treated control day 7 sample A, 6) the level of gene expression in the carotid collar-implanted, vehicle-treated control day 7 sample B, 7) the level of gene expression in the sham-operated, vehicle-treated control day 7 sample A, 8) the level of gene expression in the sham-operated, vehicle-treated control day 7 sample B, 9) the level of gene expression in the carotid collar-implanted, lercanidipine-treated day 7 sample, 10) the level of gene expression in the sham-operated, lercanidipine-treated day 7 sample A, and 11) the level of gene expression in the sham-operated, lercanidipine-treated day 7 sample B.

Additionally, several of the DSTs were further characterized as shown in Tables 2 and 3 and their nucleotide sequences are provided as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 in the Sequence Listing below.

The ligation of the sequence into a vector does not regenerate the MspI site; the experimentally determined sequence reported herein has C-G-G as the first bases of the 5' end.

The data shown in Figure 1 were generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-G-A-C-C; SEQ ID NO: 28) paired with the "universal" 3' primer (SEQ ID NO:21) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

Figure 1 is a graphical representation of the results of TOGA™ runs using a 5' PCR primer with parsing bases GACC (SEQ ID NO:28) and the universal 3' PCR primer (SEQ ID NO:21) showing PCR products produced from mRNA extracted from rabbit carotids in animals under the following conditions: carotid collar-implanted and treated with vehicle (1 day) (Panel A), carotid collar-implanted and treated with lercanidipine (1day) (Panel B), sham-operated and treated with vehicle (1 day) (Panel C), sham-operated and treated with lercanidipine (1 day) (Panel D), carotid collar-implanted and treated with vehicle (7 days) (Panel E), sham-operated and treated with vehicle (7 days) (Panel F), carotid collar-implanted and treated with lercanidipine (7 days) (Panel G), and sham-operated and treated with lercanidipine (7 days) (Panel H), where the vertical index line indicates a PCR product of about 456 base pairs (b.p.). The horizontal axis represents the number of base pairs of the

molecules in these samples and the vertical axis represents the fluorescence measurement in the TOGA™ analysis (which corresponds to the relative expression of the molecule of that address). The data presented in panels E, F, and H are plotted for independent, duplicate runs generated for these three RNA samples. The TOGA™ data indicate that the relative expression of this molecule is greatest in the sham-operated 7 day samples (Panel F & H) compared to collar-implanted day 7 samples or to the sham-operated 1 day samples.

The results of the TOGA™ runs have been normalized using the methods described in pending U.S. Patent Application Serial No. 09/318,699/U.S., and pending PCT Application Serial No. PCT/US00/14159, both entitled Methods and System for Amplitude Normalization and Selection of Data Peaks (Dennis Grace, Jayson Durham); and pending U.S. Patent Application Serial No. 09/318,679/U.S. and pending PCT Application Serial No. PCT/US00/14123, both entitled Methods for Normalization of Experimental Data (Dennis Grace, Jayson Durham) all of which are incorporated herein by reference. The vertical line drawn through the 8 panels represents the DST molecule identified as REC2_7 (SEQ ID NO:7).

Some products, which were differentially represented, appeared to migrate in positions that suggest that the products were novel based on comparison to data extracted from GenBank. The sequences of such products were determined by one of two methods: cloning or direct sequencing of the PCR products.

Cloning of TOGA™ Generated PCR Products

In suitable cases, the PCR product was isolated, cloned into a TOPO vector (Invitrogen) and sequenced on both strands. The database matches for each cloned DST sequence are listed in Table 2. DST REC2_1 (SEQ ID NO:1), the DNA molecule identified by MspI AAAC299, was one such cloned product. In order to verify that the cloned product corresponds to the TOGA™ peak of interest, the extended TOGA™ assay was performed for each DST (see below).

Direct Sequencing of TOGA™ Generated PCR Products

In other cases, the TOGA™ PCR product was sequenced using a modification of a direct sequencing methodology (Innis, *Proc Natl Acad Sci*, 1988).

PCR products corresponding to DSTs were gel purified and PCR amplified again to incorporate sequencing primers at 5' and 3' ends. The sequence addition was accomplished through 5' and 3' ds-primers containing M13 sequencing primer sequences (M13 forward

and M13 reverse respectively) at their 5' ends, followed by a linker sequence and a sequence complementary to the DST ends. Using the Clontech Taq Start antibody system, a master mix containing all components except the gel purified PCR product template was prepared, which contained sterile H_2O, 10X PCR II buffer, 10mM dNTP, 25 mM $MgCl_2$, AmpliTaq/Antibody mix (1.1 µg/µl Taq antibody, 5 U/µl AmpliTaq), 100 ng/µl of 5' ds-primer (5' TCC CAG TCA CGA CGT TGT AAA ACG ACG GCT CAT ATG AAT TAG GTG ACC GAC GGT ATC GG 3', SEQ ID NO: 23), and 100 ng/µl of 3' ds-primer (5' CAG CGG ATA ACA ATT TCA CAC AGG GAG CTC CAC CGC GGT GGC GGC C 3', SEQ ID NO: 24). After addition of the PCR template, PCR was performed using the following program: 94°C, 4 minutes and 25 cycles of 94°C, 20 seconds; 65°C, 20 seconds; 72°C, 20 seconds; and 72°C 4 minutes. The resulting amplified adapted PCR product was gel purified.

The purified PCR product was sequenced using a standard protocol for ABI 3700 sequencing. Briefly, triplicate reactions in forward and reverse orientation (6 total reactions) were prepared, each reaction containing 5 µl of gel purified PCR product as template. In addition, the sequencing reactions contained 2 µl 2.5X sequencing buffer, 2 µl Big Dye Terminator mix, 1 µl of either the 5' sequencing primer (5' CCC AGT CAC GAC GTT GTA AAA CG 3', SEQ ID NO: 25), or the 3' sequencing primer (5' TTT TTT TTT TTT TTT TTT V 3', where V=A, C, or G, SEQ ID NO: 26) in a total volume of 10 µl.

In an alternate embodiment, the 3' sequencing primer was the sequence 5' GGT GGC GGC CGC AGG AAT TTT TTT TTT TTT TTT TT 3', (SEQ ID NO: 27). PCR was performed using the following thermal cycling program: 96°C, 2 minutes and 29 cycles of 96°C, 15 seconds; 50°C, 15 seconds; 60°C, 4 minutes.

Table 2 contains the database matches for the sequences determined by this method. REC2_7 (SEQ ID NO: 7), the DNA molecule identified by MspI GACC456, was one such direct sequenced product. In order to verify that the product determined by direct sequencing corresponds to the TOGA™ peak of interest, the extended TOGA™ assay was performed for each DST (see below).

Verification Using the Extended TOGA™ Method

In order to verify that the TOGA™ peak of interest corresponds to the identified DST, an extended TOGA™ assay was performed for each DST as described below. PCR primers ("Extended TOGA™ primers") were designed from sequence determined using one of two

methods: (1) in suitable cases, the PCR product was isolated, cloned into a TOPO vector (Invitrogen) and sequenced on both strands; or (2) in other cases, the TOGA™ PCR product was sequenced using a modification of a direct sequencing methodology (Innis, *Proc Natl. Acad Sci*, 1988).

PCR was performed using the Extended TOGA™ primers and the N1 PCR reaction products as a substrate. Oligonucleotides were synthesized with the sequence G-A-T-C-G-A-A-T-C extended at the 3' end with a partial MspI site (C-G-G), and an additional 18 adjacent nucleotides from the determined sequence of the DST. For example, for the PCR product with the TOGA™ address GACC456 (DST REC2_7); SEQ ID NO:7) , the 5' PCR primer was G-A-T-C-G-A-A-T-C-C-G-G-G-A-C-C-G-A-A-A-G-C-C-A-A-A-C-G-C-A (SEQ ID NO:29). This 5' PCR primer was paired with the fluorescence labeled universal 3' PCR primer (SEQ ID NO:21) in a PCR reaction using the PCR N1 reaction product as substrate.

The length of the PCR product generated with the Extended TOGA™ primer was compared to the length of the original PCR product that was produced in the TOGA™ reaction. The results for SEQ ID NO:7, for example, are shown in Figure 2. The length of the PCR product corresponding to SEQ ID NO:7 (REC2_7) was direct sequenced and a 5' PCR primer was built from the direct sequenced DST (SEQ ID NO:29). The product obtained from PCR with this primer (SEQ ID NO:29) and the universal 3' PCR primer (SEQ ID NO:21) (as shown in the top panel) was compared to the length of the original PCR product that was produced in the TOGA™ reaction with mRNA extracted from the sham-operated and lercanidipine-treated day 7 sample using a 5' PCR primer with parsing bases GACC (SEQ ID NO:28) and the universal 3' PCR primer (SEQ ID NO:21) (as shown in the middle panel). Again, for all panels, the number of base pairs is shown on the horizontal axis, and fluorescence intensity (which corresponds to relative expression) is found on the vertical axis. In the bottom panel, the traces from the top and middle panels are overlaid, demonstrating that the peak found using an extended primer from the direct sequenced DST is the same number of base pairs as the original PCR product obtained through TOGA™ as DST REC2_7 (SEQ ID NO:7).

Sequence Identification of DSTs

Methods for alignment of biological sequences for pairwise comparison are well known in the art. Local alignments between a query sequence and a subject sequence can be derived by using the algorithm of Smith (*J Mol Biol*, 1981), by the homology alignment

algorithm of Needleman (*J Mol Biol*, 1970), or by the similarity search algorithm of Pearson (*Proc Natl Acad Sci*, 1988). A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a sequence database, can be determined using the BLAST computer program based on the algorithm of Altschul and colleagues (Altschul, *J Mol Biol*; 1990; Altschul, *Nucleic Acids Res*, 1997). The term "sequence" includes nucleotide and amino acid sequences. In a sequence alignment, the query sequence can be either protein or nucleic acid or any combination therein. BLAST is a statistically driven search method that finds regions of similarity between a query and database sequences. These are called segment pairs, and consist of gapless alignments of any part of two sequences. Within these aligned regions, the sum of the scoring matrix values of their constituent symbol pairs is higher than a level expected to occur by chance alone. The scores obtained in a BLAST search can be interpreted by the experienced investigator to determine real relationships versus random similarities. The BLAST program supports four different search mechanisms:

- **Nucleotide Query Searching a Nucleotide Database**- Each database sequence is compared to the query in a separate nucleotide-nucleotide pairwise comparison.
- **Protein Query Searching a Protein Database**- Each database sequence is compared to the query in a separate protein-protein pairwise comparison.
- **Nucleotide Query Searching a Protein Database**- The query is translated, and each of the six products is compared to each database sequence in a separate protein-protein pairwise comparison.
- **Protein Query Searching a Nucleotide Database**- Each nucleotide database sequence is translated, and each of the six products is compared to the query in a separate protein-protein pairwise comparison.

By using the BLAST program to search for matches between a sequence of the present invention and sequences in GenBank and EST databases, identities were assigned whenever possible. A portion of these results is listed in Table 2.

Categorizing differentially expressed genes in the carotid artery in response to carotid collar implantation and lercanidipine

Disease progression and identification of pathological states, as in atherosclerosis, can be characterized by patterns of gene expression. The data from Table 1 identify a group of genes whose expression is modulated in a rabbit model of intimal hyperplasia. The mRNAs detected in Table 1 whose relative levels of expression were determined by TOGA™ as

differentially expressed and whose sequence identification was completed in Tables 2 and 3 were further analyzed to categorize the patterns of regulation detected in response to carotid collar implantation and lercanidipine treatment in rabbits. Genes were organized into four distinct categories as shown in Table 3, namely, those 1) up-regulated in carotid arteries of collar-implanted animals compared to sham-operated animals (example shown in Figure 3 Panel A), 2) up-regulated in carotid arteries of collar-implanted animals compared to sham-operated animals with the collar-induced expression reversed by treatment with lercanidipine (example shown in Figure 3 Panel B), 3) down-regulated in carotid arteries of collar-implanted animals compared to sham-operated animals (example shown in Figure 3 Panel C), and 4) down-regulated in carotid arteries of collar-implanted animals compared to sham-operated animals with the collar-repressed expression reversed by treatment with lercanidipine (example shown in Figure 3 Panel D). In three of these categories (1, 2, and 3 above), mRNAs were identified by TOGA™ that had no known matches to homologs in sequence databases or whose predicted gene product encoded a protein of unknown biological function. The differentially expressed nucleotide sequences or their corresponding polypeptides, either individually or more preferably as an ensemble, can be used as a diagnostic fingerprint indicative of the status of intimal hyperplasia, or of disease progression in atherosclerosis. Such a diagnostic use can be performed with TOGA™ or other simultaneous gene expression monitoring techniques, including hybridization-based cDNA or oligonucleotide arrays and sequence tag-based methods.

The data presented in Tables 1 and 3 are useful not only in predicting which genes and pathways (see below) are subject to regulation during changes in disease state, but also provide an indication of when to therapeutically intervene in order to deter or reverse the progression of atherosclerosis disease.

Assigning biological function and significance to identified gene sequences

A complete understanding of diseases, such as atherosclerosis, and the development of new pharmaceutical intervention strategies to prevent or ameliorate disease progression will require an ability to pinpoint the cellular and molecular mechanisms responsible for the underlying pathology. Identifying the genes and regulatory pathways involved in atherosclerosis will provide a foundation for a molecular description of the disease process. As outlined above, the TOGA™ process revealed a large set of differentially expressed genes in carotid tissues of a rabbit model of intimal hyperplasia and atherosclerosis. The cloning and sequencing of the differentially expressed PCR products generated by TOGA™, use of the extended TOGA™ assay, and performance of sequence database searches for each DST

provided gene identities for 17 of these molecules, as indicated in Tables 2 and 3. In this analysis, 13 of these molecules were found to be unique and 4 were determined to multiply represent the rabbit mRNA for serum amyloid A. The biological function for 8 of the DSTs could be assigned, as the encoded protein products of these genes are known and were previously established in the literature or deduced by homology to proteins of known function in other species. The function of each these 8 DSTs is listed in Table 3. For the remaining 6 DSTs, the biological roles of the deduced protein sequences, or the corresponding sequence database matches, are not known at present and none shared significant homology with any known proteins with established biological function. These data associate a variety of cellular processes potentially affected in the carotid vessel wall with intimal hyperplasia induced by the presence of the carotid collar. In addition, lercanidipine affects on the collar-mediated changes in expression of at least 3 mRNAs indicates that calcium-responsive gene regulatory pathways may be involved in the establishment of, or response to, intimal hyperplasia.

Analysis of the known biological functions of the regulated DSTs can reveal cellular consequences of intimal hyperplasia and define roles for previously unsuspected biological processes in atherosclerosis. The substantial increase in expression of serum amyloid 3 mRNA levels by collar implantation at day 7 indicates that intimal hyperplasia activates an inflammatory acute phase response in these animals. The TOGA™ data identified two genes encoding proteins with homology to extracellular matrix proteins, fibrillin-2 and EFEMP-1, both of whose expression was down-regulated in response to collar implantation. These changes may signal an alteration in the elasticity of the arterial wall due to restriction by the collar. The identification of ALG-2 as a collar- and lercanidipine-regulated transcript implicates cell death pathways in the control of intimal hyperplasia. The ALG-2 protein is a member of a large class of calcium-binding proteins and was originally identified as an apoptosis-linked gene. The ALG-2 protein appears to be required for Fas-mediated apoptosic responses. The effectiveness of lercanidipine in blocking the collar-induced repression of ALG-2 mRNA therefore provides a pharmaceutical approach to enhance the level of ALG-2, and its actions in apoptosis, during the development of intimal hyperplasia and atherosclerosis.

The expressed genes identified herein will find application in a wide range of medical uses. For example, such uses would include compound screening uses, prognostic uses, diagnostic uses, and therapeutic uses.

Other Preferred Embodiments

Diagnosis and Treatment

Where a polynucleotide of the invention is down-regulated and exacerbates a pathological condition, such as atherosclerosis, the expression of the polynucleotide can be increased or the level of the intact polypeptide product can be increased in order to treat, prevent, ameliorate, or modulate the pathological condition. This can be accomplished by, for example, administering a polynucleotide or polypeptide of the invention (or a set of polynucleotides and polypeptides including those of the invention) to the mammalian subject.

A polynucleotide of the invention can be administered alone or with other polynucleotides to a mammalian subject by a recombinant expression vector comprising the polynucleotide. A mammalian subject can be a human, baboon, chimpanzee, macaque, cow, horse, sheep, pig, horse, dog, cat, rabbit, guinea pig, rat or mouse. Preferably, the recombinant vector comprises a polynucleotide shown in the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 or a polynucleotide which is at least 98% identical to a nucleic acid sequence shown in group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. Also, preferably, the recombinant vector comprises a variant polynucleotide that is at least 80%, 90%, or 95% identical to a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

The administration of a polynucleotide or recombinant expression vector of the invention to a mammalian subject can be used to express a polynucleotide in said subject for the treatment of, for example, atherosclerosis. Expression of a polynucleotide in target cells, including but not limited to endothelial cells, smooth muscle cells, monocytes or macrophage cells, would effect greater production of the encoded polypeptide. In some cases, where the encoded polypeptide is a nuclear protein, the regulation of other genes may be secondarily up- or down-regulated.

There are available to one skilled in the art multiple viral and non-viral methods suitable for introduction of a nucleic acid molecule into a target cell, as described above. In addition, a naked polynucleotide can be administered to target cells. Polynucleotides and recombinant expression vectors of the invention can be administered as a pharmaceutical composition. Such a composition comprises an effective amount of a polynucleotide or recombinant expression vector, and a pharmaceutically acceptable formulation agent selected for suitability with the mode of administration. Suitable formulation materials preferably are non-toxic to recipients at the concentrations employed and can modify, maintain, or preserve, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. (See Gennaro AR, *Remington's Pharmaceutical Sciences,* 1990).

The pharmaceutically active compounds (i.e., a polynucleotide or a vector) can be processed in accordance with conventional methods of pharmacy to produce medicinal agents for administration to patients, including humans and other mammals. Thus, the pharmaceutical composition comprising a polynucleotide or a recombinant expression vector may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (*e.g.*, solutions, suspensions, or emulsions).

The dosage regimen for treating a disease with a composition comprising a polynucleotide or expression vector is based on a variety of factors, including the type or severity of the atherosclerosis, the age, weight, sex, medical condition of the patient, the route of administration, and the particular compound employed. Thus, the dosage regimen may vary widely, but can be determined routinely using standard methods. A typical dosage may range from about 0.1 mg/kg to about 100 mg/kg or more, depending on the factors mentioned above.

The frequency of dosing will depend upon the pharmacokinetic parameters of the polynucleotide or vector in the formulation being used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

The cells of a mammalian subject may be transfected *in vivo*, ex vivo, or *in vitro*. Administration of a polynucleotide or a recombinant vector containing a polynucleotide to a target cell *in vivo* may be accomplished using any of a variety of techniques well known to those skilled in the art. For example, U.S. Patent No. 5,672,344 describes an *in vivo* viral-mediated gene transfer system involving a recombinant neurotrophic HSV-1 vector. The above-described compositions of polynucleotides and recombinant vectors can be transfected *in vivo* by oral, buccal, parenteral, rectal, or topical administration as well as by inhalation spray. The term "parenteral" as used herein includes, subcutaneous, intravenous, intramuscular, intrasternal, infusion techniques or intraperitoneally.

While the nucleic acids and/or vectors of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more vectors of the invention or other agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions that are given at the same time or different times, or the therapeutic agents can be given as a single composition.

Another delivery system for polynucleotides of the invention is a "non-viral" delivery system. Techniques that have been used or proposed for gene therapy include DNA-ligand complexes, adenovirus-ligand-DNA complexes, direct injection of DNA, CaPO4 precipitation, gene gun techniques, electroporation, lipofection, and colloidal dispersion (Mulligan, *Science*, 1993). Any of these methods are widely available to one skilled in the art and would be suitable for use in the present invention. Other suitable methods are available to one skilled in the art, and it is to be understood that the present invention may be accomplished using any of the available methods of transfection. Several such methodologies have been utilized by those skilled in the art with varying success (Mulligan, *Science*, 1993).

Where a polynucleotide of the invention is up-regulated and exacerbates a pathological condition in a mammalian subject, such as atherosclerosis, the expression of the polynucleotide can be blocked or reduced or the level of the intact polypeptide product can be reduced in order to treat, prevent, ameliorate, or modulate the pathological condition. This can be accomplished by, for example, the use of antisense oligonucleotides, triple helix base pairing methodology or ribozymes. Alternatively, drugs or antibodies that bind to and inactivate the polypeptide product can be used.

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either

transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of gene products of the invention in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. (See Brown, *Meth Mol Biol*, 1993; Sonveaux, *Meth Mol Biol*, 1994; Uhlmann, *Chem Rev*, 1990.)

Modifications of gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of a gene of the invention. Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred.

Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (e.g., Gee, in Huber & Carr, *Molecular and Immunologic Approaches*. Mt. Kisco, NY: Futura Publishing Co, 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent nucleotides, can provide sufficient targeting specificity for mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to

determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a polynucleotide of the invention. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. (See, e.g., Agrawal, *Trends Biotechnol*, 1992; Uhlmann, *Chem Rev*, 1990; Uhlmann, *Tetrahedron Lett*, 1987.)

Ribozymes are RNA molecules with catalytic activity. (See, e.g., Cech, *Science*, 1987; Cech, *Ann Rev Biochem*, 1990; Cech, *J Biol Chem*, 1992; Couture, *Trends Genet*, 1996.) Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (e.g., Haseloff et al., U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a polynucleotide of the invention can be used to generate ribozymes which will specifically bind to mRNA transcribed from the polynucleotide. Methods of designing and constructing ribozymes which can cleave RNA molecules in *trans* in a highly sequence specific manner have been developed and described in the art (Haseloff, *Nature*, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (e.g., Gerlach et al., EP 321,201).

Specific ribozyme cleavage sites within a RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate RNA targets also can be evaluated by testing accessibility to

hybridization with complementary oligonucleotides using ribonuclease protection assays. The nucleotide sequences shown in the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 and their complements provide sources of suitable hybridization region sequences. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease polynucleotide expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff et al., U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Production of Diagnostic Tests

Pathological conditions or susceptibility to pathological conditions, such as atherosclerosis, can be diagnosed using methods of the invention. Testing for expression of a polynucleotide of the invention or for the presence of the polynucleotide product can correlate with the severity of the condition and can also indicate appropriate treatment. For example, the presence or absence of a mutation in a polynucleotide of the invention can be determined through sequencing techniques known to those skilled in the art and a pathological condition or a susceptibility to a pathological condition is diagnosed based on

the presence or absence of the mutation. Further, an alteration in expression of a polypeptide encoded by a polynucleotide of the invention can be detected, where the presence of an alteration in expression of the polypeptide is indicative of the pathological condition or susceptibility to the pathological condition. The alteration in expression can be an increase in the amount of expression or a decrease in the amount of expression.

As an additional method of diagnosis, a first biological sample from a patient suspected of having a pathological condition, such as atherosclerosis, is obtained along with a second sample from a suitable comparable control source. A biological sample can comprise saliva, blood, cerebrospinal fluid, amniotic fluid, urine, feces, or tissue, such as gastrointestinal tissue. A suitable control source can be obtained from one or more mammalian subjects that do not have the pathological condition. For example, the average concentrations and distribution of a polynucleotide or polypeptide of the invention can be determined from biological samples taken from a representative population of mammalian subjects, wherein the mammalian subjects are the same species as the subject from which the test sample was obtained. The amount of at least one polypeptide encoded by a polynucleotide of the invention is determined in the first and second sample. The amounts of the polypeptide in the first and second samples are compared. A patient is diagnosed as having a pathological condition if the amount of the polypeptide in the first sample is greater than or less than the amount of the polypeptide in the second sample. Preferably, the amount of polypeptide in the first sample falls within the range of samples taken from a representative group of patients with the pathological condition.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

The present invention also includes a diagnostic system, preferably in kit form, for assaying for the presence of the polypeptide of the present invention in a body sample, such as brain tissue, cell suspensions or tissue sections; or a body fluid sample, such as CSF, blood, plasma or serum, where it is desirable to detect the presence, and preferably the amount, of the polypeptide of this invention in the sample according to the diagnostic methods described herein.

In a related embodiment, a nucleic acid molecule can be used as a probe (i.e., an oligonucleotide) to detect the presence of a polynucleotide of the present invention, a gene corresponding to a polynucleotide of the present invention, or a mRNA in a cell that is

diagnostic for the presence or expression of a polypeptide of the present invention in the cell. The nucleic acid molecule probes can be of a variety of lengths from at least about 10, suitably about 10 to about 5000 nucleotides long, although they will typically be about 20 to 500 nucleotides in length. The probe can be used to detect the polynucleotide through hybridization methods which are extremely well known in the art and will not be described further here.

In a related embodiment, detection of genes corresponding to the polynucleotides of the present invention can be conducted by primer extension reactions such as the polymerase chain reaction (PCR). To that end, PCR primers are utilized in pairs, as is well known, based on the nucleotide sequence of the gene to be detected. Preferably, the nucleotide sequence is a portion of the nucleotide sequence of a polynucleotide of the present invention. Particularly preferred PCR primers can be derived from any portion of a DNA sequence encoding a polypeptide of the present invention, but are preferentially from regions which are not conserved in other cellular proteins.

Preferred PCR primer pairs useful for detecting the genes corresponding to the polynucleotides of the present invention and expression of these genes are described in the TOGA™ Process Section above and in the Tables. Nucleotide primers from the corresponding region of the polypeptides of the present invention described herein are readily prepared and used as PCR primers for detection of the presence or expression of the corresponding gene in any of a variety of tissues.

In another embodiment, a diagnostic system, preferably in kit form, is contemplated for assaying for the presence of the polypeptide of the present invention or an antibody immunoreactive with the polypeptide of the present invention in a body fluid sample. Such diagnostic kit would be useful for monitoring the fate of a therapeutically administered polypeptide of the present invention or an antibody immunoreactive with the polypeptide of the present invention. The system includes, in an amount sufficient for at least one assay, a polypeptide of the present invention and/or a subject antibody as a separately packaged immunochemical reagent.

Instructions for use of the packaged reagent(s) are also typically included.

A diagnostic system of the present invention preferably also includes a label or indicating means capable of signaling the formation of an immunocomplex containing a polypeptide or antibody molecule of the present invention.

Any label or indicating means can be linked to or incorporated in an expressed protein, polypeptide, or antibody molecule that is part of an antibody or monoclonal antibody

composition of the present invention or used separately, and those atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel proteins methods and/or systems.

The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturing them to form a fluorochrome (dye) that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes such as fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), tetramethylrhodamine isothiocyanate (TRITC), lissamine, rhodamine 8200 sulphonyl chloride (RB 200 SC) and the like. A description of immunofluorescence analysis techniques is found in DeLuca, "Immunofluorescence Analysis", in *Antibody As a Tool*, Marchalonis et al., Eds., (1982), which is incorporated herein by reference. Other suitable labeling agents are known to those skilled in the art.

In preferred embodiments, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, or the like. In such cases where the principal indicating group is an enzyme such as HRP or glucose oxidase, additional reagents are required to visualize the fact that a receptor-ligand complex (immunoreactant) has formed. Such additional reagents for HRP include hydrogen peroxide and an oxidation dye precursor such as diaminobenzidine. An additional reagent useful with glucose oxidase is 2,2'-amino-di-(3-ethyl-benzthiazoline-G-sulfonic acid) (ABTS).

Radioactive elements are also useful labeling agents and are used illustratively herein. An exemplary radiolabeling agent is a radioactive element that produces gamma ray emissions. Elements which themselves emit gamma rays, such as ^{124}I, ^{125}I, ^{128}I, ^{132}I and ^{51}Cr represent one class of gamma ray emission-producing radioactive element indicating groups. Particularly preferred is ^{125}I. Another group of useful labeling means are those elements such as ^{11}C, ^{18}F, ^{15}O and ^{13}N which themselves emit positrons. The positrons so emitted produce gamma rays upon encounters with electrons present in the animal's body. Also useful is a beta emitter, such ^{111}indium or ^{3}H.

The linking of labels or labeling of polypeptides and proteins is well known in the art. For instance, antibody molecules produced by a hybridoma can be labeled by metabolic incorporation of radioisotope-containing amino acids provided as a component in the culture medium (Galfre, *Meth Enzymol*, 1981). The techniques of protein conjugation or coupling through activated functional groups are particularly applicable (Avrameas, *Scand J Immunol*, 1978; Rodwell, *Biotech*, 1984; and U.S. Patent No. 4,493,795).

The diagnostic systems can also include, preferably as a separate package, a specific binding agent. Exemplary specific binding agents are second antibody molecules, complement proteins or fragments thereof, S. aureus protein A, and the like. Preferably the specific binding agent binds the reagent species when that species is present as part of a complex.

In preferred embodiments, the specific binding agent is labeled. However, when the diagnostic system includes a specific binding agent that is not labeled, the agent is typically used as an amplifying means or reagent. In these embodiments, the labeled specific binding agent is capable of specifically binding the amplifying means when the amplifying means is bound to a reagent species-containing complex.

The diagnostic kits of the present invention can be used in an "ELISA" format to detect the quantity of the polypeptide of the present invention in a sample. A description of the ELISA technique is found in Sites, *Basic and Clinical Immunology*, 4th Ed., Chap. 22, 1982 and in U.S. Patent No. 3,654,090; U.S. Patent No. 3,850,752; and U.S. Patent No. 4,016,043, which are all incorporated herein by reference.

Thus, in some embodiments, a polypeptide of the present invention, an antibody or a monoclonal antibody of the present invention can be affixed to a solid matrix to form a solid support that comprises a package in the subject diagnostic systems.

A reagent is typically affixed to a solid matrix by adsorption from an aqueous medium, although other modes of affixation applicable to proteins and polypeptides can be used that are well known to those skilled in the art. Exemplary adsorption methods are described herein.

Useful solid matrices are also well known in the art. Such materials are water insoluble and include the cross-linked dextran available under the trademark SEPHADEX from Pharmacia Fine Chemicals (Piscataway, NJ), agarose, polystyrene beads of about 1 micron (μm) to about 5 millimeters (mm) in diameter available from several suppliers (e.g., Abbott Laboratories, Chicago, IL), polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose- or nylon-based webs (sheets, strips or paddles) or tubes, plates or the wells of a microtiter plate, such as those made from polystyrene or polyvinylchloride.

The reagent species, labeled specific binding agent, or amplifying reagent of any diagnostic system described herein can be provided in solution, as a liquid dispersion or as a substantially dry power, e.g., in lyophilized form. Where the indicating means is an enzyme, the enzyme's substrate can also be provided in a separate package of a system. A solid

support such as the before-described microtiter plate and one or more buffers can also be included as separately packaged elements in this diagnostic assay system.

The packaging materials discussed herein in relation to diagnostic systems are those customarily utilized in diagnostic systems.

Genes

The present invention also relates to the genes corresponding to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 and translations of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Homologues

Also provided in the present invention are homologues including paralogous genes and orthologous genes. Homologues may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for the desired homologue.

Polypeptides

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art. See, e.g., *Curr. Prot. Mol. Bio.*, Chapter 16.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or

leader sequences, pro-sequences, sequences which aid in purification (such as multiple histidine residues), or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified by the one-step method described in Smith (*Gene*, 1988). Polypeptides of the invention also can be purified from natural or recombinant sources using antibodies of the invention raised against the secreted protein in methods which are well known in the art.

Signal Sequences

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein (*Virus Res.*, 1985). The method of von Heijne uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein (*Nucleic Acids Res,* 1986). Therefore, from a deduced amino acid sequence, a signal sequence and mature sequence can be identified.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called Signal P (Nielsen, *Protein Engineering*, 1997), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated.

As one of ordinary skill in the art would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence corresponding to the translations of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

Polynucleotide or polypeptide variants differ from the polynucleotides or polypeptides of the present invention, but retain essential properties thereof. In general, variants have close similarity overall and are identical in many regions to the polynucleotide or polypeptide of the present invention.

Further embodiments of the present invention include polynucleotides having at least 80% identity, more preferably at least 90% identity, and most preferably at least 95%, 96%, 97%, 98% or 99% identity to a sequence contained in the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the polynucleotides having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity will encode a polypeptide identical to an amino acid sequence contained in the translations of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

Further embodiments of the present invention also include polypeptides having at least 80% identity, more preferably at least 85% identity, more preferably at least 90% identity, and most preferably at least 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence contained in translations of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. Preferably, the above polypeptides should exhibit at least one biological activity of the protein. In a preferred embodiment, polypeptides of the present invention include polypeptides having at least 90% similarity, more preferably at

least 95% similarity, and still more preferably at least 96%, 97%, 98%, or 99% similarity to an amino acid sequence contained in translations of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

Methods for aligning polynucleotides or polypeptides are codified in computer programs, including the GCG program package (Devereux, *Nuc Acids Res,* 1984), BLASTP, BLASTN, FASTA (Altschul, *J Mol Biol*; 1990), and Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) which uses the local homology algorithm of Smith (*J Mol Biol*, 1981).

When using any of the sequence alignment programs to determine whether a particular sequence is, for instance, 95% identical to a reference sequence, the parameters are set such that the percentage of identity is calculated over the full length of the reference polynucleotide and that gaps in identity of up to 5% of the total number of nucleotides in the reference polynucleotide are allowed.

A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag (*Comp App Biosci*, 1990). The term "sequence" includes nucleotide and amino acid sequences. In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is presented in terms of percent identity. Preferred parameters used in a FASTDB search of a DNA sequence to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, and Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, and Window Size=500 or query sequence length in nucleotide bases, whichever is shorter. Preferred parameters employed to calculate percent identity and similarity of an amino acid alignment are: Matrix=PAM 150, k-tuple=2, Mismatch Penalty= 1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty=0.05, and Window Size=500 or query sequence length in amino acid residues, whichever is shorter.

As an illustration, a polynucleotide having a nucleotide sequence of at least 95% "identity" to a sequence contained in the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8,

SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 means that the polynucleotide is identical to a sequence contained in the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 or the cDNA except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the total length (not just within a given 100 nucleotide stretch). In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, up to 5% of the nucleotides in the sequence contained in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17 or the cDNA can be deleted, inserted, or substituted with other nucleotides. These changes may occur anywhere throughout the polynucleotide.

Similarly, a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference polypeptide, means that the amino acid sequence of the polypeptide is identical to the reference polypeptide except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the total length of the reference polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the

degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons. For instance, a polynucleotide variant may be produced to optimize codon expression for a particular host (i.e., codons in the human mRNA may be changed to those preferred by a bacterial host, such as *E. coli*).

The variants may be allelic variants. Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (Lewin, *Genes II*, 1985). These allelic variants can vary at either the polynucleotide and/or polypeptide level. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis. See, e.g., *Curr. Prot. Mol. Bio.*, Chapter 8.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as decreased aggregation. As known, aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity (see, e.g., Pinckard, *Clin Exp Immunol,* 1967; Robbins, *Diabetes*, 1987; Cleland, *Crit Rev Therap Drug Carrier Sys*, 1993). Similarly, interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein (Dobeli, *J Biotechnology*, 1988).

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle conducted extensive mutational analysis of human cytokine IL-la (*J Biol Chem*, 1993). These investigators used random mutagenesis to generate over 3,500 individual IL- l a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators concluded that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Gayle, *J Biol Chem*, 1993). In fact, only 23 unique amino acid sequences, out of more than 3,500 amino acid sequences examined, produced a protein that differed significantly in activity from the wild-type sequence. Another experiment demonstrated that one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. Ron reported variant KGF proteins

having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues (Ron, *J Biol Chem,* 1993).

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, *Science*, 1990, wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, the amino acid positions which have been conserved between species can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions in which substitutions have been tolerated by natural selection indicate positions which are not critical for protein function. Thus, positions tolerating amino acid substitution may be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site-directed mutagenesis or alanine-scanning mutagenesis (the introduction of single alanine mutations at every residue in the molecule) can be used (Cunningham, *Science*, 1989). The resulting mutant molecules can then be tested for biological activity.

According to Bowie et al., these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, the most buried or interior (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface or exterior side chains

are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln; replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp; and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include: (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code; (ii) substitution with one or more of amino acid residues having a substituent group; (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (e.g., polyethylene glycol); (iv) fusion of the polypeptide with additional amino acids, such as an IgG Fc fusion region peptide, a leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

Polynucleotide and Polypeptide Fragments

In the present invention, a "**polynucleotide fragment**" refers to a short polynucleotide having a nucleic acid sequence contained in that shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17. The short nucleotide fragments are preferably at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in that shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17. These nucleotide fragments are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, and greater than 150 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, to the end of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17. In this context "about" includes the particularly recited ranges, larger or smaller by several nucleotides (i.e., 5, 4, 3, 2, or 1 nt) at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity.

In the present invention, a "polypeptide fragment" refers to a short amino acid sequence contained in the translations of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, or 61 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, or 60 amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several amino acids (5, 4, 3, 2, or 1) at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotide fragments encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix-forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha

amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of the translations of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotide fragments encoding these domains are also contemplated.

Other preferred fragments are biologically active fragments or the polynucleotide encoding biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Epitopes & Antibodies

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, *Proc Natl Acad Sci,* 1985), further described in U.S. Patent No. 4,631,211.

In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. (See, e.g., Wilson, *Cell*, 1984; Sutcliffe, *Science*, 1983).

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art. (See, e.g., Sutcliffe, *Science*, 1983; Wilson, *Cell*, 1984; Chow, *Proc Natl Acad Sci*, 1985; Francis, *J Gen Virol*, 1985). A preferred immunogenic epitope includes the secreted protein. The immunogenic epitope may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse). Alternatively, the immunogenic epitope may be prescribed without a carrier, if the sequence is of sufficient length (at least about 25 amino acids). However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting.)

As used herein, the term "**antibody**" (Ab) or "**monoclonal antibody**" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab and F(ab')2

fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl, *J Nucl Med*, 1983). Thus, these fragments are preferred, as well as the products of a Fab or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and human and humanized antibodies.

The antibodies may be chimeric antibodies, e.g., humanized versions of murine monoclonal antibodies. Such humanized antibodies may be prepared by known techniques, and offer the advantage of reduced immunogenicity when the antibodies are administered to humans (Co, *Nature*, 1991). In one embodiment, a humanized monoclonal antibody comprises the variable region of a murine antibody (or just the antigen binding site thereof) and a constant region derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable region fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in (Riechmann, *Nature*, 1988 ; Liu, PNAS, 1987; Chiang, *Biotechniques*, 1989; Winter, *Trends Pharmacol* Sci, 1993; Zou, *Science,* 1993; Zou, *Curr Biol*, 1994; Walls, *Nucleic Acids Res*, 1993).

One method for producing a human antibody comprises immunizing a non-human animal, such as a transgenic mouse, with a polypeptide translated from a nucleotide sequence chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, whereby antibodies directed against the polypeptide translated from a nucleotide sequence chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 are generated in said animal. Procedures have been developed for generating human antibodies in non-human animals. The antibodies may be partially human, or preferably completely human. For example, mice have been prepared in which one or more endogenous immunoglobulin genes are inactivated by various means and human immunoglobulin genes are introduced into the mice to replace the inactivated mouse genes. Such transgenic mice may be genetically altered in a variety of ways. The genetic manipulation may result in human immunoglobulin polypeptide chains replacing endogenous immunoglobulin chains in at least some (preferably

virtually all) antibodies produced by the animal upon immunization. Examples of techniques for production and use of such transgenic animals are described in U.S. Patent Nos. 5,814,318, 5,569,825, and 5,545,806, which are incorporated by reference herein. Antibodies produced by immunizing transgenic animals with a polypeptide translated from a nucleotide sequence chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 are provided herein.

Monoclonal antibodies may be produced by conventional procedures, e.g., by immortalizing spleen cells harvested from the transgenic animal after completion of the immunization schedule. The spleen cells may be fused with myeloma cells to produce hybridomas by conventional procedures. Examples of such techniques are described in U.S. Patent No. 4,196,265, which is incorporated by reference herein.

A method for producing a hybridoma cell line comprises immunizing such a transgenic animal with an immunogen comprising at least seven contiguous amino acid residues of a polypeptide translated from a nucleotide sequence chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17; harvesting spleen cells from the immunized animal; fusing the harvested spleen cells to a myeloma cell line, thereby generating hybridoma cells; and identifying a hybridoma cell line that produces a monoclonal antibody that binds a polypeptide translated from a nucleotide sequence chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. Such hybridoma cell lines, and monoclonal antibodies produced therefrom, are encompassed by the present invention. Monoclonal antibodies secreted by the hybridoma cell line are purified by conventional techniques. Examples of such techniques are described in U.S. Patent No. 4,469,630 and U.S. Patent No. 4,361,549.

Antibodies may be employed in an *in vitro* procedure, or administered *in vivo* to inhibit biological activity induced by a polypeptide translated from a nucleotide sequence chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID

NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. Disorders caused or exacerbated (directly or indirectly) by the interaction of such polypeptides of the present invention with cell surface receptors thus may be treated. A therapeutic method involves *in vivo* administration of a blocking antibody to a mammal in an amount effective for reducing a biological activity induced by a polypeptide translated from a nucleotide sequence chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. For example, antibody blockade of the LDL lipoproteins to receptors at sites of endothelium injury may inhibit the formation of atherosclerotic plaques. Similarly, antibody blockade of macrophage or platelet adhesion to endothelial lesions may block the initiation of plaque formation.

Also provided herein are conjugates comprising a detectable (e.g., diagnostic) or therapeutic agent, attached to an antibody directed against a polypeptide translated from a nucleotide sequence chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. Examples of such agents are well known, and include but are not limited to diagnostic radionuclides, therapeutic radionuclides, and cytotoxic drugs. (See, e.g., Thrush, *Annu Rev Immunol,* 1996.) The conjugates find use in *in vitro* or *in vivo* procedures.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional

regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

In addition, polypeptides of the present invention, including fragments and, specifically, epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker, *Nature*, 1988). Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone (Fountoulakis, *J Biol Chem*, 1995).

Similarly, EP A 0 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties (see, e.g., EP A 0 232 262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5 (See, Bennett, *J Mol Recognit*, 1995; Johanson, *J Biol Chem*, 1995).

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as

the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, CA), among others, many of which are commercially available. As described in Gentz, for instance, hexa-histidine provides for convenient purification of the fusion protein (*Proc Natl Acad Sci,* 1989). Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, *Cell,* 1984). Other fusion proteins may use the ability of the polypeptides of the present invention to target the delivery of a biologically active peptide. This might include focused delivery of a toxin to tumor cells, or a growth factor to stem cells.

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention. See, e.g., *Curr. Prot. Mol. Bio.*, Chapter 9.6.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells. See, e.g., *Curr. Prot. Mol. Bio.*, Chapters 9.9, 16.15.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli, Streptomyces and Salmonella*

typhimurium cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells, and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, PNH16A, PNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis, *Basic Methods in Molecular Biology,* 1995. It is specifically contemplated that the polypeptides of the present invention may, in fact, be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from products purified from natural sources, including bodily fluids, tissues and

cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells.

Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome-marking reagents based on actual sequence data (repeat polymorphisms) are presently available. Each polynucleotide of the present invention can be used as a chromosome marker. For example, defects in the gene encoding low density lipoprotein receptor would be indicative of familial hypercholesterolemia in homozygous or heterozygous individuals.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the a sequence in the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene-mapping strategies that can be used include *in situ* hybridization, prescreening with labeled flow-sorted

chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides of 2,000-4,000 bp are preferred. For a review of this technique, see Verma, *Human Chromosomes: A Manual of Basic Techniques*, 1988.

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross-hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. Disease mapping data are found, for example, in McKusick, *Mendelian Inheritance in Man*, Kruglyak, *Am J Hum Genet*, 1995; *Curr. Prot. Hum. Genet.* Assuming one megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. The polynucleotides of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 can be used for this analysis of individuals. These may be indirect, through associations with risk factors such as diabetes, or direct, through genetic defects in lipid or cholesterol metabolism. These can be used as markers to identify individuals with susceptibility to atherosclerosis.

First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations is ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from

several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Both methods rely on binding of the polynucleotide to DNA or RNA. For these techniques, preferred polynucleotides are usually 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (Lee, *Nuc Acids Res*, 1979; Cooney, *Science*, 1988; Beal, *Science*, 1991) for discussion of triple helix formation) or to the mRNA itself (Okano, *J Neurochem*, 1991; *Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression*, 1988) for a discussion of antisense technique). Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease.

Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, *J Cell Biol*, 1985; Jalkanen, *J Cell Biol*, 1987). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). See, e.g., *Curr. Prot. Mol. Bio.*, Chapter 11. Suitable antibody assay labels are known in the art and include enzyme labels, such as glucose oxidase; and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{111}In), and technetium (^{99m}Tc); fluorescent labels, such as fluorescein and rhodamine; and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected *in vivo* by imaging. Antibody labels or markers for *in vivo* imaging of protein include those detectable by X-radiography, nuclear magnetic resonance (NMR), or electron spin resonance (ESR). For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety such as a radioisotope (e.g., ^{131}I, ^{111}In, ^{99m}Tc), a radio-opaque substance, or a material detectable by NMR, is introduced (e.g., parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, the quantity of radioactivity necessary for a human subject will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in Burchiel, "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, 1982).

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder. For example, macrophages activated in atherosclerotic lesions may show specific changes in gene expression. If these changes are also found in macrophages circulating in peripheral blood, this may be detected in blood samples from patients.

Moreover, polypeptides of the present invention can be used to treat disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide; to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B); to inhibit the activity of a polypeptide (e.g., an oncogene); to activate the activity of a polypeptide (e.g., by binding to a receptor); to reduce the activity of a membrane bound receptor by competing with it for

free ligand (e.g., soluble tumor necrosis factor (TNF) receptors used in reducing inflammation); or to bring about a desired response (e.g., blood vessel growth).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor). Polypeptides can be used as antigens to trigger immune responses. For example, activated macrophages that are filled with lipid in atherosclerotic lesions may express genes unique to this activated state. Immunization against these markers may stimulate antibody responses or cellular immune responses that could eliminate the lipid-laden macrophages, and eliminate the atherosclerotic lesions.

Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. See, e.g., *Curr. Prot. Mol. Bio.*, Chapter 11.15. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Biological Activities

The polynucleotides and polypeptides of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides could be used to treat the associated disease.

Nervous System Activity

A polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the central nervous system or peripheral nervous system by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of neuroblasts, stem cells, or glial cells. Also, a polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the central nervous system or peripheral nervous system by activating or inhibiting the mechanisms of synaptic transmission, synthesis, metabolism and inactivation of neural transmitters, neuromodulators and trophic factors, and by activating or inhibiting the expression and incorporation of enzymes, structural proteins, membrane channels, and receptors in neurons and glial cells.

The etiology of these deficiencies or disorders may be genetic, somatic (such as cancer or some autoimmune disorder), acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotide or polypeptide of the present invention can be used as a marker or detector of a particular nervous system disease or disorder. The disorder or disease can be any of Alzheimer's disease, Pick's disease, Binswanger's disease, other senile dementia, Parkinson's disease, parkinsonism, obsessive compulsive disorders, epilepsy, encephaolopathy, ischemia, alcohol addiction, drug addiction, schizophrenia, amyotrophic lateral sclerosis, multiple sclerosis, depression, and bipolar manic-depressive disorder. Alternatively, the polypeptide or polynucleotide of the present invention can be used to study circadian variation, aging, or long-term potentiation, the latter affecting the hippocampus. Additionally, particularly with reference to mRNA species occurring in particular structures within the central nervous system, the polypeptide or polynucleotide of the present invention can be used to study brain regions that are known to be involved in complex behaviors, such as learning and memory, emotion, drug addiction, glutamate neurotoxicity, feeding behavior, olfaction, viral infection, vision, and movement disorders.

Immune Activity

A polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic (such as cancer or some autoimmune disorders) acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotide or polypeptide of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

A polynucleotide or polypeptide of the present invention may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. A polypeptide or polynucleotide of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Di George's Syndrome, HIV infection, HTLV-BLV infection, leukocyte

adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, a polypeptide or polynucleotide of the present invention could also be used to modulate hemostatic (bleeding cessation) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, a polynucleotide or polypeptide of the present invention could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, a polynucleotide or polypeptide of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment of heart attacks (infarction), strokes, or scarring.

A polynucleotide or polypeptide of the present invention may also be useful in the treatment or detection of autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of a polypeptide or polynucleotide of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, or in some way results in the induction of tolerance, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected by the present invention include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by a polypeptide or polynucleotide of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

A polynucleotide or polypeptide of the present invention may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of a polypeptide or polynucleotide of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, a polypeptide or polynucleotide of the present invention may also be used to modulate inflammation. For example, the polypeptide or polynucleotide may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

Hyperproliferative Disorders

A polypeptide or polynucleotide can be used to treat or detect hyperproliferative disorders, including neoplasms. A polypeptide or polynucleotide of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, a polypeptide or polynucleotide of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by inducing the proliferation, differentiation, or mobilization of T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as by administering the polypeptide or polynucleotide as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but are not limited to neoplasms located in the abdomen, bone, breast, digestive system, liver, pancreas,

peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic region, skin, soft tissue, spleen, thoracic region, and urogenital system.

Similarly, other hyperproliferative disorders can also be treated or detected by a polynucleotide or polypeptide of the present invention. Examples of such hyperproliferative disorders include, but are not limited to hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

Infectious Disease

A polypeptide or polynucleotide of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, the polypeptide or polynucleotide of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide of the present invention. Examples of viruses include, but are not limited to, the following DNA and RNA viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza), Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to, arthritis, bronchiollitis, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g.,

Kaposi's, warts), and viremia. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but are not limited to, the following Gram-Negative and Gram-positive bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, Enterobacteriaceae (Klebsielia, Salmonella, Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Menigococcal), Pasteurellacea infections (e.g., Actinobacillus, Heamophilus, Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, and Staphylococcus. These bacterial or fungal families can cause numerous diseases or symptoms, including, but not limited to, bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections (such as whooping Cough or empyema), sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis, Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually-transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, and wound infections. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Moreover, parasitic agents causing disease or symptoms that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but are not limited to, the following families: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas. These parasites can cause a variety of diseases or symptoms, including, but not limited to, Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), Malaria, pregnancy complications, and toxoplasmosis. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Preferably, treatment using a polypeptide or polynucleotide of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (*ex vivo* therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

A polynucleotide or polypeptide of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues (Frontiers in Medicine, *Science*, 1997). The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g., osteoporosis, osteocarthritis, periodontal disease, liver failure), surgery (including cosmetic plastic surgery), fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vascular (including vascular endothelium), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, ligament) tissue. Preferably, regeneration occurs without scarring or with minimal scarring. Regeneration also may include angiogenesis. In the case of atherosclerosis, improper healing of vascular endothelium lesions may be the primary trigger of atherosclerotic plaque formation. Molecules that may induce more efficient wound healing may prevent plaque formation.

Moreover, a polynucleotide or polypeptide of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. A polynucleotide or polypeptide of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with

peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotide or polypeptide of the present invention.

Chemotaxis

A polynucleotide or polypeptide of the present invention may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide of the present invention may increase chemotaxic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that a polynucleotide or polypeptide of the present invention may inhibit chemotactic activity. Such molecules could also be used to treat a variety of disorders. Thus, a polynucleotide or polypeptide of the present invention could be used as an inhibitor of chemotaxis. Blockade of this step may prevent development of atherosclerotic plaques.

Atherosclerotic plaque develops over several decades and involves inflammatory cell infiltration, smooth muscle cell proliferation, accumulation of extracellular matrix, fibrous cap formation, and angiogenesis (Bayes-Genis, *Circ Res,* 2000). Chemotaxis is involved in the early development of atherosclerosis. Cell populations migrate toward the inner part of the vascular wall and originate the neointima, which leads to the formation of an atherosclerotic plaque. For example, monocyte chemotaxis is induced by monocyte chemoattractant protein 1 (MCP-1), which is expressed early in the development of atherosclerosis in the injured arterial wall. (Furukawa, *Circ Res,* 1999; Han, *J Lipid Res,* 1999). Additionally, insulin-like growth factors (IGF) have been shown to promote macrophage chemotaxis and also stimulate vascular smooth muscle proliferation and

migration to form the neointima (Bayes-Genis, *Circ Res,* 2000). Activated platelets and C-reactive protein are important in inducing a significant increase in MCP-1 and recruiting monocytes, respectively (Gawaz, *Atherosclerosis,* 2000; Torzewski, *Arterioscler Thromb Vasc Biol,* 2000). Furthermore, vascular endothelial growth factor (VEGF) has been shown to be a critical regulator of angiogenesis that stimulates proliferation, migration and proteolytic activity of endothelial cells. VEGF is able to stimulate chemotaxis in monocytes and can enhance matrix metalloproteinase expression and accelerate smooth muscle cell migration (Wang, *Circ Res* 1998). Blockade of one or more of these chemotaxic activities may prevent development of atherosclerotic plaques.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (i.e., an agonist), increase, inhibit (i.e., an antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic (Coligan, Chapter 5. in *Current Protocols in Immunology,* 1991). Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds or, at least, related to a fragment of the receptor capable of being bound by the polypeptide (e.g., an active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or *E. coli.* Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product

mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues. At present, many of the diagnostic tools are only able to identify risk factors for atherosclerosis (e.g., hyperlipidemia), and do not indicate the presence of actively developing atherosclerotic plaques. New assays using markers generated from materials of the present invention may provide some specific indicators of active disease.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the invention, (b) assaying a biological activity, and (c) determining if a biological activity of the polypeptide has been altered.

Other Activities

A polypeptide or polynucleotide of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells from a lineage other than the above-described hemopoietic lineage.

A polypeptide or polynucleotide of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide or polynucleotide of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide or polynucleotide of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, circadian rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, the response to opiates and opioids, tolerance to opiates and opioids, withdrawal from opiates and opioids, reproductive capabilities (preferably by activin or inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

A polypeptide or polynucleotide of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors, or other nutritional components.

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Table 1

Seq ID NO	DST ID	Digital Address (MspI)		Control Collar day 1	lercani-dipine collar day 1	control sham day 1	lercani-dipine sham day 1	control collar day 7 duplicate A	control collar day 7 duplicate B	control sham day 7 duplicate A	control sham day 7 duplicate B	lercani-dipine collar day 7	lercani-dipine sham day 7 duplicate A	lercani-dipine sham day 7 duplicate B
1	REC2_1	AAAC	299	89	124	84	59	197	215	59	19	285	76	26
		AACT	98	629	209	179	469	255	234	591	334	153	408	634
		AATA	232	253	75	155	169	697	609	757	559	834	737	796
		ACCA	381	45	39	59	54	36	62	81	69	422	85	50
		ACCC	314	868	733	346	782	1116	1132	1340	147	244	224	1048
		ACCG	160	1529	1303	1447	534	1824	1079	1021	355	251	681	554
2	REC2_2	ACCT	273	236	252	265	560	172	129	247	299	22	270	164
3	REC2_3	ACGC	369	31	22	25	20	114	83	16	20	132	22	15
		AGCC	182	1544	363	485	168	1867	1780	1089	1340	2225	1029	1070
		AGCC	379	80	24	49	23	184	167	226	232	125	233	206
		AGCG	290	213	126	126	203	484	255	173	48	75	174	297
4	REC2_4	AGGA	277	159	115	86	122	311	262	73	101	367	108	86
		AGTT	413	62	52	65	66	153	97	133	109	27	108	190
5	REC2_5	CACT	98	5940	4379	3496	5824	774	693	4894	3978	638	2981	5576
		CCCT	401	546	601	606	192	214	275	264	164	61	128	169
		CCTA	285	314	101	168	171	295	184	82	231	38	49	19
		CGAG	340	1832	157	185	105	3023	3935	4186	3278	3270	2500	4349
		CGAT	223	75	16	26	31	89	52	148	92	104	91	142
		CGAT	345	369	100	90	168	205	221	171	381	145	614	335
		CGCA	291	262	86	169	267	99	203	101	102	123	102	93
		CGGA	211	148	123	179	56	16	23	114	52	77	170	110
		CTGA	216	260	251	230	174	61	65	130	104	197	85	82
		CTGA	289	2228	334	353	297	2096	2140	1371	1348	1607	1523	1273
		CTGT	84	94	46	68	60	325	328	233	606	94	403	297
		CTTA	211	81	69	31	106	61	84	105	83	482	122	82
		GAAT	173	198	44	173	63	321	360	474	209	495	452	152
6	REC2_6	GACC	333	17	22	32	20	71	50	122	107	26	105	138
7	REC2_7	GACC	456	35	54	58	65	80	50	203	243	33	239	188

Table 1 (continued)

Seq ID NO	DST ID	Digital Address (MspI)	Control Collar day 1	lercani-dipine collar day 1	control sham day 1	lercani-dipine sham day 1	control collar day 7 duplicate A	control collar day 7 duplicate B	control sham day 7 duplicate A	control sham day 7 duplicate B	lercani-dipine collar day 7	lercani-dipine sham day 7 duplicate A	lercani-dipine sham day 7 duplicate B
8	REC2 8	GATC 109	100	92	133	81	832	745	401	234	1253	276	385
		GCCC 398	119	96	65	61	300	401	576	483	97	410	384
		GCGC 236	383	118	358	347	1224	676	773	795	997	864	895
		GCGC 397	36	37	41	37	126	145	185	203	32	206	149
9	REC2 9	GCGT 197	76	100	130	32	1664	1414	95	78	103	147	80
10	REC2 10	GCTG 338	60	45	106	50	103	160	23	33	148	68	57
		GGCT 226	305	82	95	68	303	270	243	206	224	285	260
11	REC2 11	GTCC 204	2646	1359	773	896	257	238	421	356	318	467	475
		TACA 226	478	131	154	454	607	748	406	497	563	373	424
12	REC2 12	TATG 97	231	429	380	438	1101	1210	431	315	1608	235	423
13	REC2 13	TCAA 450	141	109	113	280	152	164	373	490	577	536	417
		TCGG 272	122	115	127	67	423	404	258	71	121	102	357
14	REC2 14	TCGG 366	34	32	36	172	156	132	345	593	37	429	296
		TCTC 336	821	196	282	394	673	70	57	109	361	59	188
15	REC2 15	TCTC 419	312	389	386	1022	129	306	700	1040	502	932	469
		TGAA 376	53	81	65	159	44	37	47	91	152	183	64
		TGAG 173	483	153	295	254	513	377	421	293	360	305	776
16	REC2 16	TGAG 339	23	31	31	25	205	440	122	129	316	80	74
17	REC2 17	TGCT 238	274	182	260	641	296	254	796	814	290	864	989
		TGGA 168	241	158	144	289	174	209	203	125	809	129	267
		TTCT 452	89	139	88	85	232	178	283	261	734	322	378
		TTTA 163	400	85	174	175	136	133	199	120	187	173	210

Table 2

Seq ID NO	DST ID	Digital Address (MspI)	Database Match (Accession #)	% Identity	Nucleotide homology	
					DST nucleotide range (bp#)	Database nucleotide range (bp#)
1	REC2_1	AAAC 299	Rabbit serum amyloid A (SAA3) mRNA, complete cds (M64696.1)	100%	3 – 178	296 – 471
2	REC2_2	ACCT 273	NOVEL	N/A	N/A	N/A
3	REC2_3	ACGC 369	Rabbit serum amyloid A (SAA3) mRNA, complete cds. (M64696.1)	99%	3 – 249	225 – 471
4	REC2_4	AGGA 277	Rabbit serum amyloid A (SAA3) mRNA, complete cds (M64696.1)	99%	3 – 156	318 – 471
5	REC2_5	CACT 98	NOVEL	N/A	N/A	N/A
6	REC2_6	GACC 333	Homology to xd90a02.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:2604842 3 similar to gb:U03272 FIBRILLIN 2 (HUMAN) (AW119195.1)	94%	157 – 283	3 – 129
7	REC2_7	GACC 456	Homology to S.scrofa LPL mRNA for lipoprotein lipase (triacyl-glycero-protein acylhydrolase) (X62984.1)	85%	23 – 391	1244 – 1612
8	REC2_8	GATC 109	Homology to homo sapiens, NADH:ubiquinone oxidoreductase MLRQ subunit homolog, clone MGC:19921 IMAGE:4138587, mRNA, complete cds.(BC011910)	100%	2 – 58	1144 – 1200
9	REC2_9	GCGT 197	EST mv37a02.x1 GuayWoodford Beier mouse kidney day 0 Mus musculus cDNA clone IMAGE:657194 3', mRNA sequence (AI464504.1)	89%	1 – 138	29 – 167
10	REC2_10	GCTG 338	Rabbit serum amyloid A (SAA3) mRNA, complete cds. (M64696.1)	98%	2 – 217	256 – 471
11	REC2_11	GTCC 204	NOVEL	N/A	N/A	N/A
12	REC2_12	TATG 97	NOVEL	N/A	N/A	N/A
13	REC2_13	TCAA 450	Homo sapiens ALG-2 interacting protein 1 (AIP1) mRNA, complete cds.(AF151793)	87%	68 – 400	2886 – 3219

Table 2 (Continued)

Seq ID NO	DST ID	Digital Address (Msp1)	Database Match (Accession #)	% Identity	Nucleotide homology	
					DST nucleotide range (bp#)	Database nucleotide range (bp#)
14	REC2_14	TCGG 366	Homo sapiens EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1) mRNA (NM_004105.1)	92%	2 – 295	1130 – 1423
15	REC2_15	TCTC 419	Homo sapiens small nuclear ribonucleoprotein polypeptide E (SNRPE) mRNA (M37716)	93%	10 – 220	136 – 346
16	REC2_16	TGAG 339	Homology to Human ribosomal protein S21 (RPS21) mRNA, complete cds (L04483.1)	87%	5 – 234	57 – 286
17	REC2_17	TGCT 238	NOVEL	N/A	N/A	N/A

EST = Expressed Sequence Tag; N/A = Not Applicable

TABLE 3

DST	MspI address	Con collar day 1	Ler collar day 1	Con collar day 7	Ler collar day 7	gene identity	biological function
UP-REGULATED IN COLLAR COMPARED TO SHAM							
REC2_1	AAAC 299	ND	ND	UP	UP	serum amyloid A (SAA3)	acute-phase apolipoprotein
REC2_3	ACGC 369	ND	ND	UP	UP	serum amyloid A (SAA3)	acute-phase apolipoprotein
REC2_4	AGGA 277	ND	ND	UP	UP	serum amyloid A (SAA3)	acute-phase apolipoprotein
REC2_10	GCTG 338	ND	ND	UP	UP	serum amyloid A (SAA3)	acute-phase apolipoprotein
REC2_8	GATC 109	ND	ND	UP	UP	homologous to NADH:ubiquinone oxidoreductase MLRQ subunit	oxidative phosphorylation
REC2_16	TGAG 339	ND	ND	UP	UP	homologous to ribosomal protein S21 (RPS21)	protein synthesis
REC2_12	TATG97	ND	ND	UP	UP	novel	unknown
UP-REGULATED IN COLLAR COMPARED TO SHAM AND REVERSED BY LERCANIDIPINE							
REC2_9	GCGT 197	ND	ND	UP	ND	novel	unknown
REC2_11	GTCC204	UP	ND	ND	ND	novel	unknown
DOWN-REGULATED IN COLLAR COMPARED TO SHAM							
REC2_5	CACT 98	ND	ND	DOWN	DOWN	novel	unknown
REC2_6	GACC 333	ND	ND	DOWN	DOWN	homologous to fibrillin-2	extracellular matrix protein
REC2_7	GACC 456	ND	ND	DOWN	DOWN	homologous to lipoprotein lipase	lipid metabolism
REC2_17	TGCT 238	ND	ND	DOWN	DOWN	novel	unknown
REC2_14	TCGG 366	ND	ND	DOWN	DOWN	homologous to EGF-containing fibulin-like extracellular matrix protein 1	extracellular matrix protein
REC2_2	ACCT 273	ND	ND	DOWN	DOWN	novel	unknown
REC2_15	TCTC 419	ND	ND	DOWN	DOWN	homologous to small nuclear ribonucleoprotein polypeptide E (SNRPE)	RNA splicing
DOWN-REGULATED IN COLLAR COMPARED TO SHAM AND REVERSED BY LERCANIDIPINE							
REC2_13	TCAA450	ND	ND	DOWN	ND	homologous to ALG-2	calcium-binding protein

CLAIMS

We Claim:

1. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

2. The method of Claim 1, wherein the medical condition is atherosclerosis.

3. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

4. The method of Claim 3, wherein the medical condition is atherosclerosis.

5. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of an antibody that binds specifically to a polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

6. The method of Claim 5, wherein the medical condition is atherosclerosis.

7. The method of Claim 6, wherein the antibody is a monoclonal antibody.

8. The method of Claim 6, wherein the antibody is a polyclonal antibody.

9. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective

amount of an antibody that binds specifically to a fragment of a polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

10. The method of Claim 9, wherein the medical condition is atherosclerosis.

11. The method of Claim 10, wherein the antibody is a monoclonal antibody.

12. The method of Claim 10, wherein the antibody is a polyclonal antibody.

13. A method of diagnosing a medical condition in a subject comprising:

(a) determining the presence or absence of a mutation in one or more of the polynucleotides chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17; and

(b) diagnosing the medical condition based on the presence or absence of said mutation.

14. The method of Claim 13, wherein the medical condition is atherosclerosis.

15. A method of diagnosing a medical condition or a susceptibility to a medical condition in a subject comprising detecting an alteration in expression of a polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, wherein the presence of an alteration in expression of the polypeptide is indicative of the medical condition or susceptibility to the medical condition.

16. The method of Claim 15, wherein the alteration in expression is an increase in the amount of expression or a decrease in the amount of expression.

17. The method of Claim 15, wherein the medical condition is atherosclerosis.

18. The method of Claim 17, wherein the method further comprises the steps of:

(a) obtaining a first biological sample from a patient suspected of having atherosclerosis and obtaining a second sample from a suitable comparable control source;

(b) determining the amount of at least one polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 in the first and second sample; and

(c) comparing the amount of the polypeptide in the first and second samples;

(d) wherein a patient is diagnosed as having atherosclerosis if the amount of the polypeptide in the first sample is greater than or less than the amount of the polypeptide in the second sample.

19. A method for using a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 for the manufacture of a medicament for the treatment of atherosclerosis.

20. A method for using a polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 for the manufacture of a medicament for the treatment of atherosclerosis.

21. A method for using an antibody that binds specifically to a polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 for the manufacture of a medicament for the treatment of atherosclerosis.

22. A method for using a gene corresponding to a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 for the manufacture of a medicament for the treatment of atherosclerosis.

23. A method for identifying a binding partner to a polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 comprising:

(a) contacting said polypeptide with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

24. An isolated gene corresponding to the cDNA sequence of a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

25. A method of using an isolated gene corresponding to the cDNA sequence of a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 for the treatment of atherosclerosis.

26. A method of identifying an activity of an expressed polypeptide in a biological assay, wherein the method comprises:

(a) expressing a polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 in a cell;

(b) isolating the expressed polypeptide;

(c) testing the expressed polypeptide for an activity in a biological assay; and

(d) identifying the activity of the expressed polypeptide based on the test.

28. A kit for detecting the presence of a polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 in a mammalian tissue sample comprising a first antibody which immunoreacts with a mammalian protein encoded by a gene corresponding to said polynucleotide or with the polypeptide encoded by said polynucleotide in an amount sufficient for at least one assay and suitable packaging material.

29. The kit of Claim 28, further comprising a second antibody that binds to the first antibody.

30. The kit of Claim 29, wherein the second antibody is labeled.

31. The kit of Claim 30, wherein the label comprises enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, or bioluminescent compounds.

32. A kit for detecting the presence of a gene encoding an protein comprising a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, or fragment thereof having at least 10 contiguous bases, in an amount sufficient for at least one assay, and suitable packaging material.

33. A method for detecting the presence of a nucleic acid molecule encoding a protein in a mammalian tissue sample, comprising the steps of:

(a) hybridizing a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and

SEQ ID NO:17 or fragment thereof having at least 10 contiguous bases, with the nucleic acid of the sample; and

(b) detecting the presence of the hybridization product.

34 A substantially pure isolated DNA molecule suitable for use as a probe for genes regulated in atherosclerosis, chosen from the group consisting of the DNA molecules identified in Table 1, having a 5' partial nucleotide sequence and length as described by their digital address, and having a characteristic regulation pattern in atherosclerosis.

35. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of two or more polynucleotides chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

36. The method of Claim 35, wherein said medical condition is atherosclerosis.

37. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of polypeptides encoded by two or more polynucleotides chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

38. The method of Claim 37, wherein said medical condition is atherosclerosis.

39. A method for assessing the stage of atherosclerosis, or assessing the efficacy of a therapeutic treatment, by testing for regulation of a panel of at least two polynucleotides drawn from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

40. A method of identifying biomolecules associated with atherosclerosis comprising the steps of:

(a) developing a cellular experiment for atherosclerosis;

(b) harvesting the RNA from the cells used in the experiment;

(c) obtaining a gene expression profile; and

(d) using the gene expression profile for identifying biomolecules which expression was altered during the experiment.

41. The method of Claim 40, wherein the biomolecules identified are polynucleotides.

42. The method of Claim 40, wherein the biomolecules identified are polypeptides.

43. A method of assessing the efficacy or toxicity of a therapeutic treatment by testing for regulation of a polynucleotide or panel of polynucleotides chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

44. The method of Claim 43, wherein the therapeutic treatment is for atherosclerosis.

45. A method of using a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 for the delivery of genes, DNA vaccines, diagnostic reagents, peptides, proteins, or macromolecules.

46. A method of identifying an activity of an expressed polypeptide in a biological assay, wherein the method comprises:

(a) expressing a polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID

NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 in a cell;

(b) isolating the expressed polypeptide;

(c) testing the expressed polypeptide for activity in a biological assay;

(d) and identifying the activity of the expressed polypeptide based on the test results.

47. A method of diagnosing or monitoring the effects of treating a subject with a dihydropyridine calcium antagonist comprising detecting an alteration in expression of at least one polypeptide encoded by at least one polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

48. The method of Claim 47, wherein the dihydropyridine calcium antagonist is lercanidipine.

49. An isolated polynucleotide and the complements and degenerate variants thereof chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having a nucleotide sequence.

50. An isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having an amino acid sequence.

51. A polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID

NO:17 and the complements and degenerate variants thereof wherein the expression of said polypeptide is upregulated or downregulated in atherosclerosis.

52. An isolated nucleic acid molecule at least ten bases in length that is hybridizable to a hybridization probe the nucleotide sequence which consists of the coding sequences of an isolated polynucleotide and the complements and degenerate variants thereof chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 under stringent conditions.

53. An isolated nucleic acid molecule comprising a sequence at least 95% identical to an isolated polynucleotide and the complements and degenerate variants thereof chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

54. An isolated nucleic acid molecule comprising a species homologue to an isolated polynucleotide and the complements and degenerate variants thereof chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

55. An antisense polynucleotide to an isolated polynucleotide and the complements and degenerate variants thereof chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

56. The antisense polynucleotide of Claim 55 wherein the polynucleotide comprises at least three nucleotides of an isolated polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID

NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

57. An isolated nucleic acid molecule encoding a fragment of an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen fom the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having an amino acid sequence.

58. An isolated nucleic acid molecule encoding a polypeptide epitope of an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen fom the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having an amino acid sequence.

59. An isolated nucleic acid molecule encoding a species homologue of an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen fom the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having an amino acid sequence.

60. A recombinant vector comprising an isolated polynucleotide and the complements and degenerate variants thereof chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having a nucleotide sequence.

61. A recombinant host cell comprising an isolated polynucleotide and the complements and degenerate variants thereof chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ

ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having a nucleotide sequence.

63. The recombinant host cell of Claim 61, comprising vector sequences.

64. A recombinant host cell that expresses an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen fom the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having an amino acid sequence.

65. The isolated polypeptide produced by the steps of:

(a) culturing a recombinant host cell that expresses an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having an amino acid sequence, under conditions such that said polynucleotide is expressed;

(b) isolating the polypeptide;

(c) using the polypeptide for diagnosing, treating, modulating or ameliorating atherosclerosis.

66. An isolated polypeptide comprising an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having an amino acid sequence, with conservative amino acid substitutions.

67. An isolated polypeptide comprising a variant of an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID

NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, which has the same biological activity as a polypeptide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

68. An isolated polypeptide comprising a species homologue of the polypeptide of an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

69. An isolated polynucleotide wherein the nucleotide sequence comprises the nucleotide sequence of an isolated polynucleotide and the complements and degenerate variants thereof chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having a nucleotide sequence with sequential nucleotide deletions from either the 5' end or the 3'end, or internal deletions.

70. An isolated polypeptide wherein the amino acid sequence comprises the amino acid sequence of an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having an amino acid sequence with sequential amino acid deletions from either the C-terminus or the N-terminus or internal deletions.

71. An isolated polypeptide, the amino acid sequence of which comprises at least ten consecutive residues of an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID

NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having an amino acid sequence and wherein said polypeptide has biological activity.

72. A purified polypeptide, the amino acid sequence of which comprises a sequence at least 90% identical to an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

73. An isolated antibody that binds specifically to an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

74. The isolated antibody of Claim 73, wherein the antibody is a monoclonal antibody.

75. The isolated antibody of Claim 73, wherein the antibody is a polyclonal antibody.

76. An isolated antibody that binds specifically to a fragment of an isolated polypeptide and the complements and degenerate variants thereof encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

77. The isolated antibody of Claim 76, wherein the antibody is a monoclonal antibody.

78. The isolated antibody of Claim 76, wherein the antibody is a polyclonal antibody.

79. The isolated gene corresponding to the cDNA sequence of an isolated polynucleotide and the complements and degenerate variants thereof chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 having a nucleotide sequence.

80. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist used to control gene expression.

81. The method of Claim 80, wherein the dihydropyridine calcium channel antagonist is lercanidipine.

82. The method of Claim 80, wherein the medical condition is atherosclerosis.

83. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that decreases expression of the polynucleotide of SEQ ID NO:9 and SEQ ID NO:11 and increases the expression of the polynucleotide of SEQ ID NO:13.

84. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that decreases the expression of the polypeptide encoded by the polynucleotide of SEQ ID NO:9 and SEQ ID NO:11 and increases the expression of the polypeptide encoded by the polynucleotide of SEQ ID NO:13.

85. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that decreases the expression of the polynucleotide of SEQ ID NO:9 and SEQ ID NO:11.

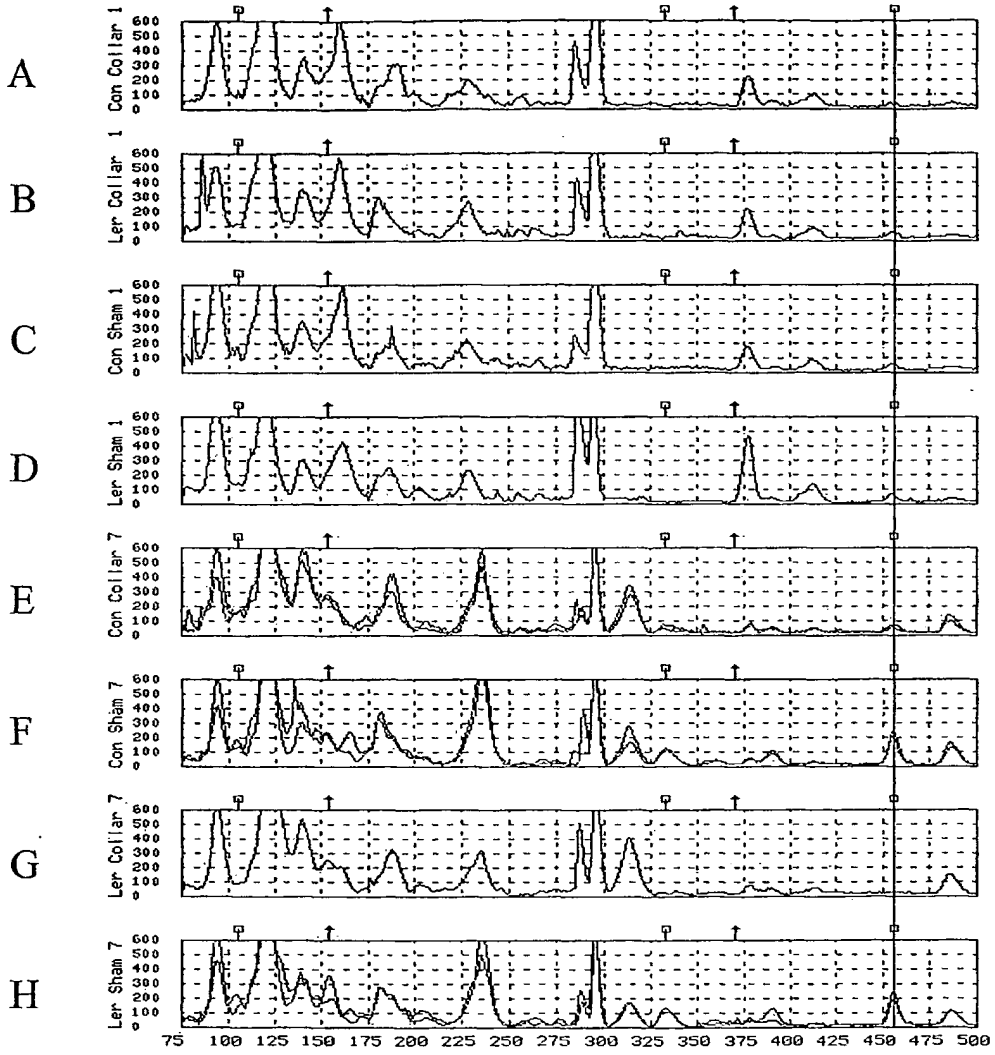
86. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that decreases the expression of the polypeptide encoded by the polynucleotide of SEQ ID NO:9 and SEQ ID NO:11.

87. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that increases the expression of the polynucleotide of SEQ ID NO:13.

88. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of a dihydropyridine calcium channel antagonist that increases the expression of the polypeptide encoded by the polynucleotide of SEQ ID NO:13.

89. The method of Claim 88, wherein the medical condition is atherosclerosis.

90. The method of Claim 88, wherein the dihydropyridine calcium channel antagonist is lercanidipine.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G
H
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Ler Sham 7
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200
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Figure 1

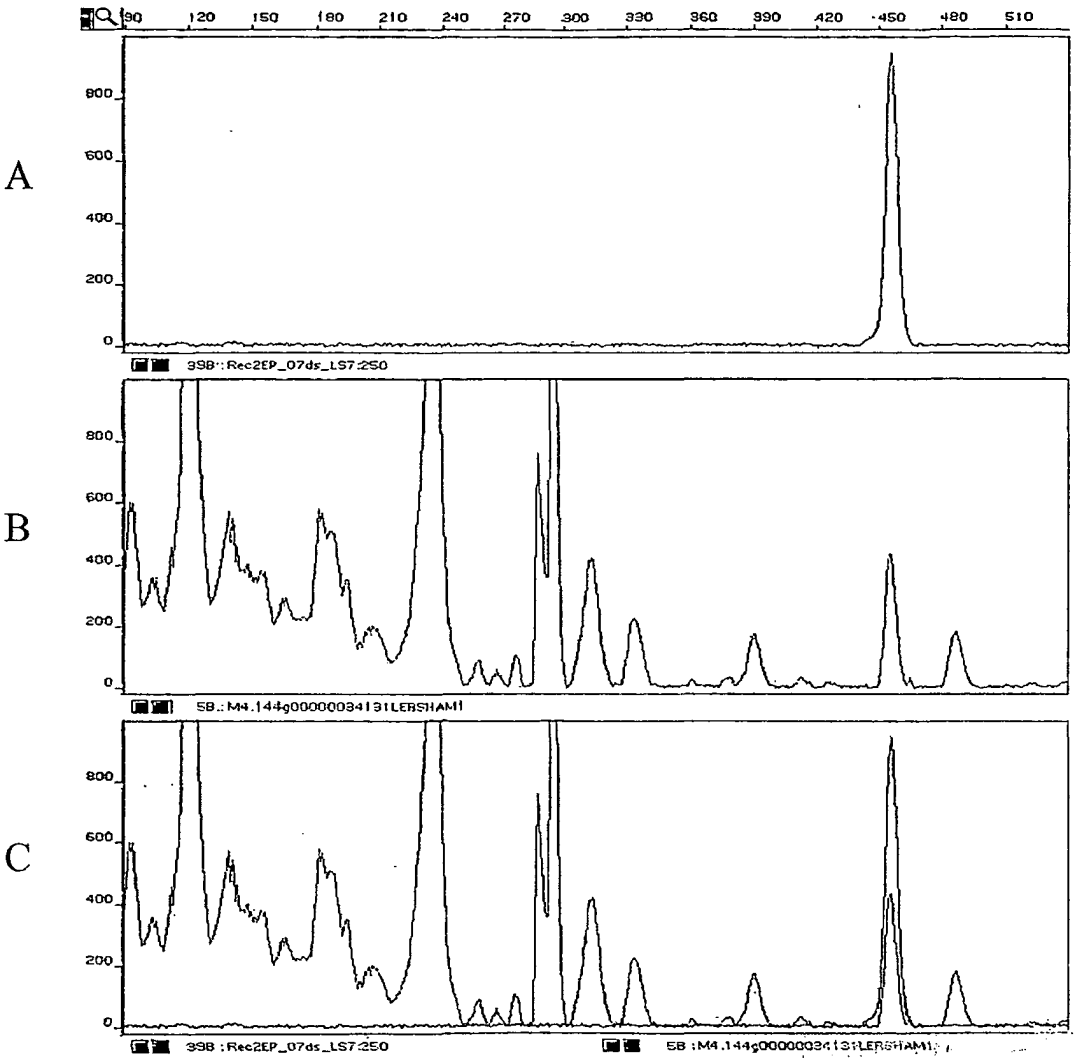
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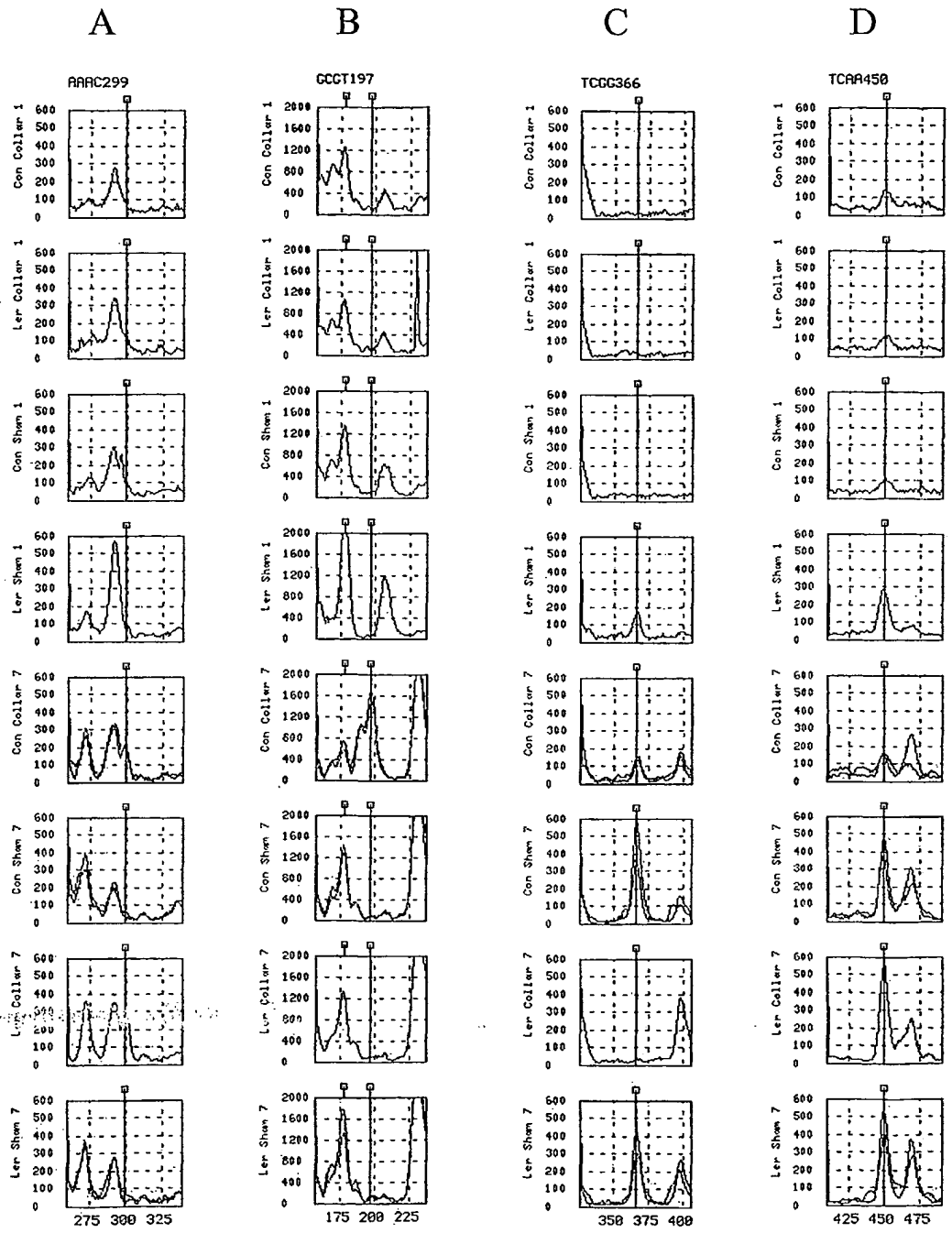
Figure 2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D
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TCAA450
Con Collar 1
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Con Sham 1
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Ler Collar 7
Ler Sham 7
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350 375 400
425 450 475

Figure 3

SEQUENCE LISTING

<110> Leonardi, Amedeo

Sartani, Abraham

Glass, James R.

Sutcliffe, J. Gregor

Hasel, Karl W.

<120> Modulation of Gene Expression During Intimal Hyperplasia of the Carotid Artery

<130> REC2

<140> 60/252,216

<141> 2000-11-21

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<170> PatentIn version 3.1

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gctccctgct cccaccgcgc tcaggagaac tgagcctgct gggacactgc tgaagtagaa 180

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tcaaaaa 247

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ccaacctatg gggccgcagt ggcaacgacc ccaatcactt cagacctaag ggcctgcccg 180

acaaatattg agctccctgc tcccaccgcg ctcaggagaa ctgagcctgc tgggacactg 240

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cttcctcatc tacacggaag tggacattgg ggagttgctg atgatgaaac tcaagtggaa 180

cagcgactcc tacttcagct gggccgactg gtggggcagc cccagcttca ccattgcgaa 240

gatcagggtg aaggcaggag agactcagaa aaaggtgatc ttctgctcca gggagaaagt 300

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aatcacttca gacctaaggg cctgcccgac aaatattgag ctccctgctc ccaccgcgct 180

caggagaact gatcctgctg ggacactgct gaagtagaat tctgtatctc tagcacttgg 240

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cactaagtat aataggattt gaaataaatt aagttcctaa aagttgaaaa atgagaaatt 180

aaacctgcaa gcaaagtatt tgaaattact attctttcta cagaagacat gggtggacat 240

caggtactta taaaaatggt ttaagtactg atattcaaga aacctaagtt ttctttctcc 300

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cctgtcagtg caatgcttgt gctcgtgaag tcactgacag gacccagaga ctacatcgtg 180

gatctggaga tgctgacagt caacagtata gggaccttcc gcacaagctc ggtgttgcga 240

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ggacgactcc attctgcggc tggccaagtc cgacggcatc gtctcgcaga acttctgagc 240

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